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Cynthia Adaku Chilaka

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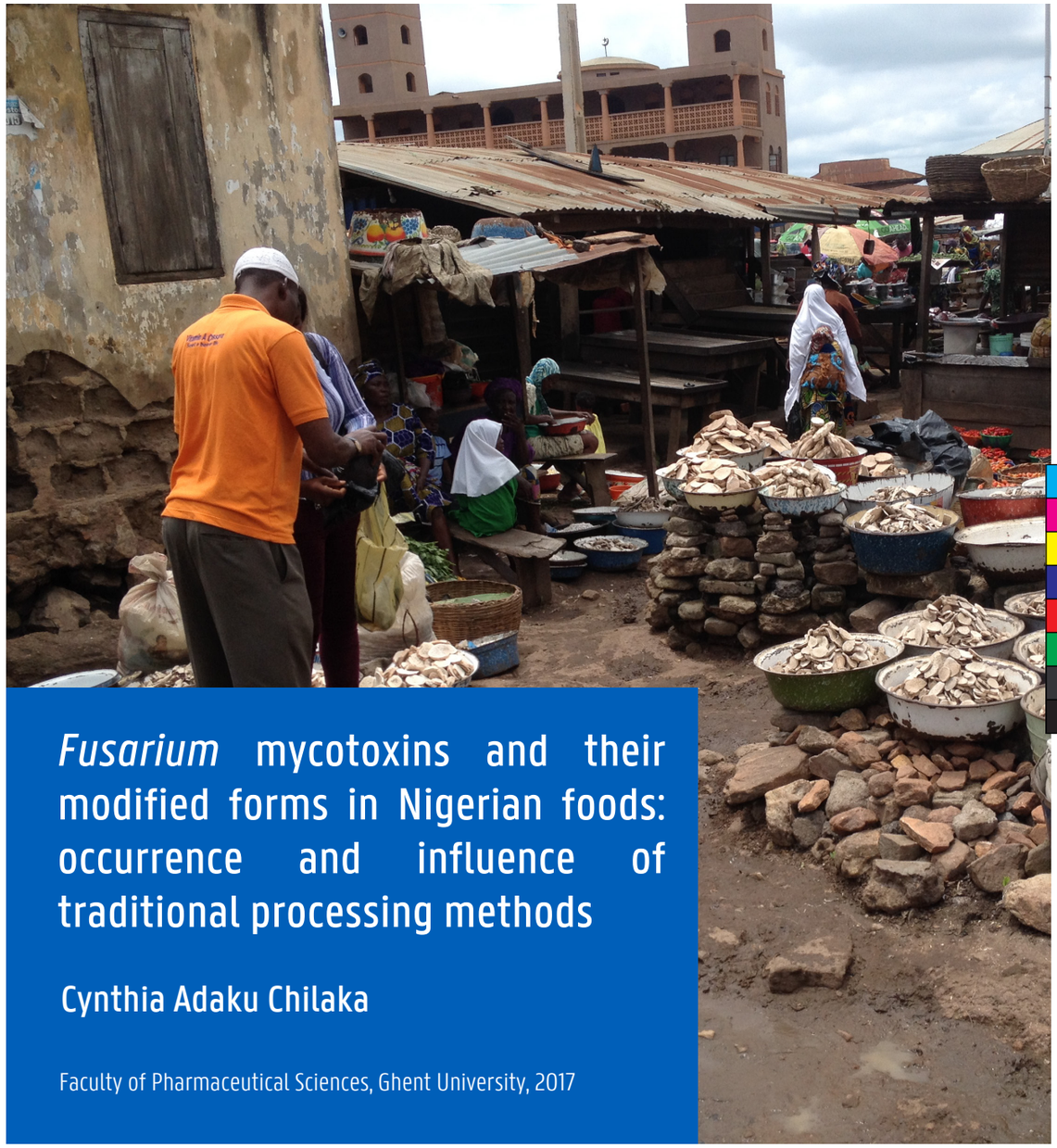

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Faculty of Pharmaceutical Sciences, Ghent University, 2017

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FUSARIUM MYCOTOXINS AND THEIR MODIFIED FORMS IN NIGERIAN FOODS: OCCURRENCE AND INFLUENCE OF TRADITIONAL PROCESSING METHODS

Cynthia Adaku Chilaka

2017

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Picture of a local market in Ayetoro, Ekiti State, Nigeria, courtesy of Dr. J. E. Obidiegwu.

"I can do all things through Christ which strengtheneth me".

Philippians 4:13

DEDICATION

DEDICATION

To my best friend and mentor, Dr. Jude Ejikeme Obidiegwu, thanks for believing in me.

&

To my late mother, Mrs Rosanna Ihuoma Chilaka Nwoko, may your soul rest in Peace, Amen.

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TABLE OF CONTENTS

TABLE OF CONTENTS

DEDICATION v
ACKNOWLEDGEMENTS vii
TABLE OF CONTENTS x
LIST OF FIGURES xv
LIST OF TABLES xix
LIST OF ABBREVIATIONS xxii
CHAPTER 1: GENERAL INTRODUCTION 1
1.1 Introduction 3
1.2 *Fusarium* mycotoxins: origin, chemistry, and distribution 5
1.2.1 Fumonisins 5
1.2.2 Trichothecenes 7
1.2.3 Zearalenone 9
1.2.4 Emerging and modified *Fusarium* mycotoxins 11
1.3 Regulation of *Fusarium* mycotoxins 18
1.4 *Fusarium* mycotoxins in sub-Saharan Africa 21
1.5 Mitigation strategies of *Fusarium* mycotoxins during processing 24
1.5.1 Cleaning and milling 24
1.5.2 Thermal treatment 25
1.5.3 Fermentation 26
1.6 Analytical techniques for determination of mycotoxins 28
1.6.1 Sample extraction and clean-up method 28
1.6.2 Detection methods 30
1.7 Conclusion 32
1.8 References 33
CHAPTER 2: STUDY RATIONALE AND OBJECTIVES 51
2.1 Nigeria: study location 53
2.2 Study Rationale 55
2.3 References 57
Chapter 3: OCCURRENCE OF *FUSARIUM* MYCOTOXINS IN CEREALS AND CEREAL-BASED PRODUCTS FROM NIGERIA 59
3.1 Introduction 61
3.2 Materials and methods 62
3.2.1 Sampling 62
3.2.2 Chemicals and reagents 65
3.2.3 Sample extraction 66
3.2.4 Preparation of the hydrolysed FB_1, FB_2, and FB_3 standard 66
3.2.5 Sample preparation for hydrolysed fumonisins 68
3.2.6 Liquid chromatography-tandem mass spectrometry 68

3.2.7 Method validation....69
3.2.8 Statistical analysis....69
3.3 Results and discussion....72
3.3.1 *Fusarium* mycotoxin contamination in cereals (maize, sorghum, and millet) and processed products (*ogi, burukutu*, and *pito*) from Nigeria....72
3.3.2. Fumonisin and modified fumonisin contamination in cereals (maize, sorghum, and millet) and processed products (*ogi*) from Nigeria....77
3.3.3 Distribution of *Fusarium* mycotoxins in major cereals across the different agro-ecological zones of Nigeria....79
3.3.4 Co-occurrence of *Fusarium* mycotoxins in cereals and processed products (*ogi, burukutu,* and *pito*) from Nigeria....83
3.4 Conclusions....85
3.5 References....86
CHAPTER 4: PREVALENCE OF *FUSARIUM* MYCOTOXINS IN ROOTS AND TUBER DERIVED PRODUCTS FROM NIGERIAN MARKETS....91
4.1 Introduction....93
4.2 Materials and methods....94
4.2.1. Sample collection....94
4.2.2 Chemical and Reagents....94
4.2.3. Mycotoxin Analysis....94
4.3 Results and discussion....97
4.4. Conclusion....101
4.5. References....102
CHAPTER 5: QUANTIFICATION OF *FUSARIUM* MYCOTOXINS IN NIGERIAN INDIGENOUS BEANS, SOYBEAN, AND THEIR PROCESSED PRODUCTS USING MULTI-MYCOTOXIN LC-MS/MS METHOD....105
5.1 Introduction....107
5.2 Materials and methods....108
5.2.1. Sample collection....108
5.2.2. Chemical and Reagents....109
5.2.3. Mycotoxin Analysis....109
5.2.4. Data Analysis....111
5.3 Results and discussion....111
5.3.1. Occurrence of *Fusarium* mycotoxins in soybean and processed soybean powder....111
5.3.2 Occurrence of *Fusarium* mycotoxins in traditional spices and indigenous beans....115

5.4 Conclusion....121
5.5 References....122
CHAPTER 6: STABILITY OF FUMONISIN B_1, DEOXYNIVALENOL, ZEARALENONE, AND T-2 TOXIN DURING PROCESSING OF TRADITIONAL BEERS AND SPICES....125
6.1. Introduction....127
6.2 Materials and Methods....128
6.2.1 Chemicals and reagents....128
6.2.2 Sample collection....128
6.2.3. Processing of traditional spices (*dawadawa, okpehe*, and *ogiri*)....128
6.2.4. Processing of traditional beers (*burukutu* and *pito*)....130
6.2.5. Sample preparation and clean-up....132
6.2.6 Liquid chromatography-tandem mass spectrometry....132
6.2.7. Statistical analysis....132
6.3. Results and discussion....133
6.3.1. Effect of traditional processing of spices on *Fusarium* mycotoxins....133
6.3.2. Effect of traditional processing of beers on *Fusarium* mycotoxins....135
6.3.2.1 Malting process....135
6.3.2.2 Brewing process....137
6.4. Conclusion....139
6.5. References....140
CHAPTER 7: INFLUENCE OF PROCESSING METHODS OF NIGERIAN TRADITIONAL INFANT FOODS (*OGI* AND SOYBEAN POWDER) ON *FUSARIUM* MYCOTOXINS....143
7.1. Introduction....145
7.2. Materials and methods....146
7.2.1. Chemicals and reagents....146
7.2.2 Origin of maize and processing of *ogi*....146
7.2.3. Origin of soybean and processing of soybean powder....147
7.2.4. Laboratory-scale production process of *ogi* and soybean powder with spiked maize and soybean grains....148
7.2.5. Sample extraction and mycotoxin analysis....149
7.2.6. Statistical analysis....149
7.3. Results and discussions....150
7.3.1. Influence of *ogi* processing method on *Fusarium* mycotoxins....150

7.3.2. Influence of the soybean powder processing method on *Fusarium* mycotoxins....156
7.4. Conclusion....162
7.5. References....163
GENERAL CONCLUSION....167
BROADER INTERNATIONAL CONTEXT, RELEVANCE AND FUTURE PERSPECTIVES....171
Broader international context....173
Relevance....176
Future perspectives....178
Reference....182
SUMMARY/ SAMENVATTING....183
Summary....185
Samenvatting....189
ANNEXES....193
CURRICULUM VITAE....221

LIST OF FIGURES

LIST OF FIGURES

CHAPTER 1: GENERAL INTRODUCTION

Figure 1.1. Chemical structure of fumonisins....5

Figure 1.2. Chemical structure of trichothecenes....8

Figure 1.3 Chemical structure of zearalenone....10

Figure 1.4 Chemical structure of fusaproliferin and deacetyl-fusaproliferin....11

Figure 1.5 Chemical structure of beauvericin and enniatins....12

Figure 1.6 The chemical structure of moniliformin. X = H (free acid), Na (sodium salt) or K (potassium salt)....12

Figure 1.7 The chemical structure of modified fumonisins (Hydrolysed fumonisin B_1, *N*-(1-deoxy-D-fructos-1-yl)-fumonisin B_1, *N*-(carboxymethyl) fumonisin B_1)....13

Figure 1.8 The chemical structure of modified trichothecenes and zearalenone....14

CHAPTER 2: STUDY RATIONALE AND OBJECTIVES

Figure 2.1. Map of Nigeria showing the different agroecological zones and all the sampling states considered in this Phd study....54

Chapter 3: OCCURRENCE OF FUSARIUM MYCOTOXINS IN CEREALS AND CEREAL-BASED PRODUCTS FROM NIGERIA

Figure 3.1. Sampling locations of maize, sorghum, millet, *burukutu, pito*, and *ogi* according to the different agro-ecological zones....63

Figure 3.2. Chromatograms showing quantifying and qualifying ions obtained with a mixture of hydrolysed fumonisin B_1 (1), B_2 (2), and B_3 (3) standard....67

Figure 3.3. Mean concentration of free and hidden fumonisins in different food types: maize, sorghum, millet, and *ogi*....78

Figure 3.4. Percentage incidence rate of *Fusarium* mycotoxins in Nigerian cereals (maize, sorghum, and millet) from different agro-ecological zones. FB_1 = fumonisin B_1, DON = deoxynivalenol, DAS = diacetoxyscirpenol....80

Figure 3.5. Differences in *Fusarium* mycotoxins in maize ((A) fumonisin B_1; and (B) diacetoxyscirpenol); and millet ((C) fumonisin B_1; and (D) diacetoxyscirpenol) across agro-ecological zones. Agro-ecological zones with the same lower-case letter are not significantly different ($P \leq 0.05$) according to Kruskal Wallis test....82

Figure 3.6 Percentage of co-occurrence of *Fusarium* mycotoxins in cereals (maize, sorghum, and millet) and cereal-based products (*ogi, burukutu,* and *pito*) from Nigerian markets....83

CHAPTER 4: PREVALENCE OF *FUSARIUM* MYCOTOXINS IN ROOTS AND TUBER DERIVED PRODUCTS FROM NIGERIAN MARKETS

Figure 4.1 Distribution of number of *Fusarium* mycotoxins in cassava and yam products from Nigeria....100

CHAPTER 5: QUANTIFICATION OF *FUSARIUM* MYCOTOXINS IN NIGERIAN INDIGENOUS BEANS, SOYBEAN, AND THEIR PROCESSED PRODUCTS USING MULTI-MYCOTOXIN LC-MS/MS METHOD

Figure 5.1 Incidence of *Fusarium* mycotoxins in soybean and processed soybean powder (PSP) samples from Nigeria. FB_1, B_2, B_3 = fumonisin B_1, B_2, B_3; ∑FB = fumonisin B_1 + B_2 + B_3; DON = deoxynivalenol; 15-ADON = 15-acetyl-DON; DON-3G = DON-3-glucoside; ZEN = zearalenone; α-ZEL = α-zearalenol; DAS = diacetoxyscirpenol; T-2 = T-2 toxin; and HT-2 = HT-2 toxin.....112

CHAPTER 6: STABILITY OF FUMONISIN B_1, DEOXYNIVALENOL, ZEARALENONE, AND T-2 TOXIN DURING PROCESSING OF TRADITIONAL BEERS AND SPICES

Figure 6.1 Schematic flow diagram of the traditional processing of *dawadawa* (A) and *ogiri* (B) showing the different sampling stages.....129

Figure 6.2 Schematic flow diagram of the traditional processing of *okpehe* showing the different sampling stages.....130

Figure 6.3 Schematic flow diagram of the traditional processing of *pito and burukutu* showing the different sampling stages.....131

Figure 6.4. The average cumulative percentage reduction of regulated *Fusarium* mycotoxins (DON = deoxynivalenol, FB_1 = fumonisin B_1, T-2 = T-2 toxin, and ZEN = zearalenone) during processing of traditional spices with traditional beans. A = African mesquite beans: *okpehe* (M2-after boiling for 12 h, M3-after dehulling, M4-after fermentation); B = African castor beans: *ogiri* (C2-after boiling for 3h, C3-after fermentation); C = African locust beans: *dawadawa* (L2-after 1st boiling, L3-after dehulling, L4-after 2nd boiling, L5-after fermentation). Sampling was conducted in triplicate and results are presented in dry weight basis.....133

Figure 6.5. (a) *Fusarium* mycotoxins concentration (µg/kg) remaining after each step during malting of sorghum, (b) average cumulative percentage reduction of regulated *Fusarium* mycotoxins during malting of sorghum. S1 - spiked sorghum, S2 – after steeping, S3 – after germination, S4 – after drying/milling. Sampling was performed in triplicate and results are presented in dry weight basis.....136

Figure 6.6. (a) *Fusarium* mycotoxins concentration (µg/kg) and (b) total amount of *Fusarium* mycotoxins remaining after each step during brewing of *burukutu.* S4 – milled malted fraction, S5 -mashed fraction, S6 – fraction after 1st boiling, S7 – sieved fraction, S8 – fraction after 1st fermentation, S9 – fraction after 2nd boiling, S10 – fraction after 2nd fermentation (*burukutu*).....137

CHAPTER 7: INFLUENCE OF PROCESSING METHODS OF NIGERIAN TRADITIONAL INFANT FOODS (*OGI* AND SOYBEAN POWDER) ON *FUSARIUM* MYCOTOXINS

Figure 7.1. Schematic flow diagram of the production process of *ogi* showing the different sampling points (M).....147

Figure 7.2. Schematic flow diagram of the production process of soybean using two different methods, and showing the different sampling points (S).....148

Figure 7.3. Concentrations (µg/kg) of fumonisin B_1 (FB_1), deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2) in the different processing steps of *ogi* (M1 = maize, M2 = 36 h fermented maize, M3 = 72 h

fermented maize, M4 = milled maize, M5 = *ogi*). Concentrations were calculated on dry weight basis. Experiments were performed in triplicate.....151

Figure 7.4. Average cumulative reduction of regulated *Fusarium* mycotoxins (FB_1 = fumonisin B_1, DON = deoxynivalenol, ZEN = zearalenone, and T-2 = T-2 toxin) during processing of *ogi* with spiked maize grains (A: 1000 µg/kg, B: 500 µg/kg). Experiments were performed in triplicate.....152

Figure 7.5. Concentrations (µg/kg) of fumonisin B_1 (FB_1), deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2) in the different processing steps of soybean powder (S1 = soybean, S2 = soaked bean, S3 = blanched bean, S4 = blanched soy powder, S6 = roasted soybean, S7 = roasted soybean powder). A = results from the roasting method, B = results from the blanching method. Experiments were performed in triplicate. Concentrations were calculated on dry weight basis.....157

Figure 7.6. Average cumulative percentage reduction of regulated *Fusarium* mycotoxins (fumonisin B_1 (FB_1), deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2)) during processing of soybean powder with spiked soybeans using two processing methods. A: roasting method/soybean spiked at 1000 µg/kg; B: roasting method/soybean spiked at 500 µg/kg; C: blanching method/soybean spiked at 1000 µg/kg; D: blanching method/soybean spiked at 500 µg/kg. Experiments were performed in triplicate.....158

LIST OF TABLES

LIST OF TABLES

CHAPTER 1: GENERAL INTRODUCTION

Table 1.1 Systematic definition of modified mycotoxins....15

Table 1.2 European Union regulatory limits (No 1881/2006) and indicative levels (No 2013/165) for *Fusarium* mycotoxins in foodstuffs....20

Chapter 3: OCCURRENCE OF *FUSARIUM* MYCOTOXINS IN CEREALS AND CEREAL-BASED PRODUCTS FROM NIGERIA

Table 3.1. Sample distribution of food commodities (maize, sorghum, millet, *burukutu, pito*, and *ogi*) from various agro-ecological zones of Nigeria....64

Table 3.2 Intraday repeatability (RSD_r) and interday reproducibility (within-laboratory) (RSD_R) of the individual mycotoxins for maize, sorghum, and millet....70

Table 3.3 Limits of detection (LOD) and limits of quantification (LOQ) of the individual mycotoxins for maize, sorghum, millet, *ogi*, *burukutu*, and *pito*....71

Table 3.4 Mean and maximum concentration (µg/kg) of *Fusarium* mycotoxins found in cereals (maize, sorghum, and millet) from Nigeria....74

Table 3.5 Mean and maximum concentration (µg/kg) of *Fusarium* mycotoxins found in cereal-based products (*ogi, burukutu,* and *pito*) from Nigeria....75

Table 3.6 Contamination levels of fumonisins, total fumonisins, and modified fumonisins in selected samples....78

Table 3.7. *Fusarium* mycotoxins occurrence in cereals (maize, sorghum, and millet) across the different agro-ecological zones of Nigeria....81

CHAPTER 4: PREVALENCE OF *FUSARIUM* MYCOTOXINS IN ROOTS AND TUBER DERIVED PRODUCTS FROM NIGERIAN MARKETS

Table 4.1. LC-MS/MS method performance characteristics of cassava (*Garri*, *Lafun*) and yam products (*Amala)*....95

Table 4.2 Intraday repeatability (RSD_r) and interday reproducibility (within-laboratory) (RSD_R) of the individual mycotoxins for cassava (*Garri*, *Lafun*) and yam products (*Amala)*....96

Table 4.3 Occurrence and levels of *Fusarium* mycotoxins (µg/kg) in cassava (*garri*, *lafun*) and yam (*amala*) products from some Nigerian markets....98

CHAPTER 5: QUANTIFICATION OF *FUSARIUM* MYCOTOXINS IN NIGERIAN INDIGENOUS BEANS, SOYBEAN, AND THEIR PROCESSED PRODUCTS USING MULTI-MYCOTOXIN LC-MS/MS METHOD

Table 5.1 Sampling sites of indigenous beans, soybean, and their processed products from Nigerian markets....109

Table 5.2. Limits of detection (LOD) and limits quantification (LOQ) of the *Fusarium* mycotoxins as obtained in the LC-MS/MS analysis of indigenous beans, traditional spices, and soybean....110

Table 5.3 Percentage of positive samples, concentration range, mean concentration, and median concentration (µg/kg) of *Fusarium* mycotoxins in soybean and processed soybean powder from the Nigerian markets....113

Table 5.4 Co-occurrence of *Fusarium* mycotoxins in soybean and processed soybean powder samples from Nigeria....115

Table 5.5 Percentage of positive samples, concentration range, mean concentration, and median concentration (µg/kg) of *Fusarium* mycotoxins in indigenous bean samples from Nigeria....117

Table 5.6 Percentage of positive samples, concentration range, mean concentration, and median concentrations (µg/kg) of *Fusarium* mycotoxins in traditional spices from Nigeria....118

Table 5.7 Co-occurrence of *Fusarium* mycotoxins in traditional spices and indigenous beans samples from Nigeria....120

CHAPTER 6: STABILITY OF FUMONISIN B1, DEOXYNIVALENOL, ZEARALENONE, AND T-2 TOXIN DURING PROCESSING OF TRADITIONAL BEERS AND SPICES

Table 6.1. Concentration (µg/kg) of *Fusarium* mycotoxins analysed by LC-MS/MS in raw beans and sorghum spiked with 500 µg/kg and 1000 µg/kg of FB_1, DON, T-2, and ZEN, respectively....129

Table 6.2 Levels of modified *Fusarium* mycotoxins detected along the process chain of traditional beer (*burukutu*)....138

CHAPTER 7: INFLUENCE OF PROCESSING METHODS OF NIGERIAN TRADITIONAL INFANT FOODS (*OGI* AND SOYBEAN POWDER) ON *FUSARIUM* MYCOTOXINS

Table 7.1. *Fusarium* mycotoxin concentration (µg/kg) in processing intermediates and products collected during *ogi* production with naturally contaminated maize....150

Table 7.2 Concentrations (µg/kg) of *Fusarium* mycotoxins detected in process intermediates and final products during the *ogi* production process....153

Table 7.3. *Fusarium* mycotoxin concentration (µg/kg) in processing intermediates collected during soybean powder production with naturally contaminated soybean using two processing methods (roasting and blanching) 156

Table 7.4 Concentrations (µg/kg) of *Fusarium* mycotoxins detected in process intermediates and final products during processing of soybeans spiked with 1000 µg/kg using two different methods (roasting and blanching) 160

LIST OF ABBREVIATIONS

LIST OF ABBREVIATIONS

Abbreviation	Definition
3-ADON	3-acetyldeoxynivalenol
15-ADON	15-acetyldeoxynivalenol
15-MAS	15-monoacetoxyscirpenol
α-ZEL	α-zearalenol
α-ZEL-14G	α-zearalenol-14-glucoside
β-ZEL	β-zearalenol
β-ZEL-14G	β-zearalenol -14-glucoside
A.	*Aspergillus*
ACB	African castor bean
AEZs	agro-ecological zones
AFB_1	aflatoxin B_1
ALB	African locust bean
AMS	African mesquite bean
APCI	atmospheric pressure chemical ionization
APPI	atmospheric pressure photo ionization
ASEAN	Association of Southeast Asian Nations
BEA	beauvericin
CAC	Codex Alimentarius Commission
CC	column chromatography
DAS	diacetoxyscirpenol
DOM	de-epoxy deoxynivalenol
DON	deoxynivalenol
DON-3G	deoxynivalenol-3-glucoside
DON-3/8/15-GLU	deoxynivalenol-3/8/15-glucuronide
DS	derived savanna
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
ENN	enniatin
ESI	electrospray ionisation
EU	European Union
F.	*Fusarium*
FA	fusaric acid
FAO	Food and Agriculture Organisation
FBs	fumonisins
FB_1	fumonisin B_1
FB_2	fumonisin B_2
FB_3	fumonisin B_3
FD	fluorescence detection
FID	flame ionization detection
FUS-X	fusarenon X
FUS	fusaproliferin
GC-MS	gas chromatography/mass spectrometry
GAP	good agricultural practices

Abbreviation	Definition
GC	gas chromatography
GDP	gross domestic product
GMP	good manufacturing practices
HACCP	hazard analysis and critical control points
HPLC	high-performance liquid chromatography
HPLC/ESI-MS/MS	liquid chromatography/electrospray ionization tandem mass spectrometry
HR-MS	high-resolution mass spectrometry
HT-2	HT-2 toxin
HT-2G	HT-2 toxin-glucoside
HT-2-3G	HT-2 toxin-3-glucoside
HT-2-4G	HT-2 toxin-4-glucoside
HT2-3/4-GLU	HT-2 toxin-3/4-glucuronide
HF	humid forest
HYFB	hydrolysed FB
$HYFB_1$	hydrolysed FB_1
IAC	immunoaffinity column,
IARC	International Agency for Research on Cancer
IITA	International Institute of Tropical Agriculture
ILSI	International Life Science Institute
IUF	immune-ultrafiltration
JECFA	Joint Expert Committee on Food Additives
KEBS	Kenya Bureau of Standards
L.	lactobacillus
LAB	Lactic acid bacteria
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LFIA	lateral flow immunoassay
LLE	liquid–liquid extraction
LOQ	limit of quantification
MA	mid altitude
MERCOSUR	Mercado Cómun del Sur
MIPs	molecularly imprinted polymers
MON	moniliformin
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MS	mass spectrometry
na	not applicable
NAFDAC	National Agency for Food and Drug Administration and Control
nd	not detected
NEO	neosolaniol
NGS	northern guinea avanna
NIV	nivalenol
NPFSIS	National Policy on Food Safety and Implementation Strategy
PACA	Partnership for Aflatoxin Control in Africa
PSP	processed soybean powder
QuEChERS	quick, easy, cheap, effective, rugged, and safe method
RASFF	rapid alert system for food and feed
S.	*Saccharomyces*

Abbreviation	Definition
SABS	South African Bureau of Standards
SaS	sahel savanna
SAX	strong anion exchange
SD	standard deviation
SFE	supercritical fluid extraction
SGS	southern guinea savanna
SPME	solid phase micro extraction
SPE	solid phase extraction
SS	sudan savanna
SSA	sub-Saharan Africa
T-2	T-2 toxin
T-2G	T-2 glucoside
T-2T	T-2 tetraol
TH	trichothecenes
TLC	thin layer chromatography
TOF	time of flight
UHPLC/TOFMS	ultra-high performance liquid chromatography/time-of-flight mass spectrometry
UN	United Nations
UV	Ultra-violet
USDA-ARS	United States Department of Agriculture, Agricultural Research Service
WHO	World Health Organisation
ZEN	zearalenone
ZEN-4S	zearalenone-4-sulphate
ZEN-14G	zearalenone-14-glucoside
ZEN-16G	zearalenone-16-glucoside
ZEN-14S	zearalenone-14-sulphate

GENERAL INTRODUCTION

The content of Chapter One is adapted from:

Chilaka CA; De Boevre M; Atanda O and De Saeger S (2017). The Status of *Fusarium* mycotoxins in sub-Saharan Africa: a review of emerging trends and post-harvest mitigation strategies towards food control. Toxins. Vol. 9: 19; doi:10.3390/toxins9010019.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Occurrence of toxigenic moulds and their toxic metabolites in food commodities is one of the major food safety concerns worldwide, but perhaps may have existed since the origin of mankind. This came into limelight owing to the outbreak of several epidemics, including ergotism, alimentary toxic aleukia (ATA), stachybotryotoxicosis, and aflatoxicosis which led to deaths of humans and animals [1]. Consequently, these events initiated a new dimension of scientific investigation. Several moulds do exist in nature, however, those of international significance include *Fusarium, Aspergillus*, *Penicillium,* and *Alternaria* because of their frequency and ability to infest various food commodities. Besides, these fungal genera have the potential to synthesise several toxic secondary metabolites, termed mycotoxins. Although, the production of mycotoxins by these moulds are not vital for their growth and reproduction, mycotoxins play an important role in their host-pathogen relationship while providing protection against fungivores and other environmental stresses [2]. Among these fungal genus, *Fusarium* is one of the most important filamentous pathogenic ascomycete mould genera widely distributed around the world. These moulds are often referred to as field or soil fungi because of their pathogenic potential, thus causing a wide range of plant diseases called fusariosis, such as vascular wilts, seedling blights, rots, and cankers to many field, horticultural, ornamental, and forest crops in both agricultural and natural ecosystems [3–5]. Fusariosis causes enormous economic losses to crop thereby affecting trade and marketing worldwide. This is evidenced by the estimated crop yield reduction between 10% and 40% reported by Bottalico and Perrone [6]. In the USA, the genus *Fusarium* has been estimated to cause losses worth of 2,900 million US dollars annually for wheat and barley [7].

In addition to their pathogenicity to plants, *Fusarium* species are capable of synthesising a wide range of toxic secondary metabolites of diverse structures and actions. Species such as *F. verticillioides* and *F. graminearum*, each have the ability to synthesise more than one metabolite. *Fusarium* metabolites of economic importance include fumonisins, zearalenone, and trichothecenes. Their importance is partly ascribed to the presence of some baseline scientific data as well as documented significant impact on public health and animal productivity across several countries. These toxins have been implicated to cause several devastating diseases in humans and animals ranging from acute to chronic with carcinogenic, oestrogenic, mutagenic, hepatotoxic, teratogenic, haemorrhagic, neurotoxic and/or immunosuppressive effects [8]. They may co-exist in feeds, foods, and processed food products, because

some fungi have the ability to produce more than one mycotoxin and/or more than one fungi species may colonise a substrate. Thus, an intrinsic quality is the exhibition of synergistic, additive, and/or antagonist health effects on the human or animal host [9]. In addition to their harmful significances to health, mycotoxins are major food contaminants affecting global food security, especially in the developing countries. The Food and Agriculture Organization (FAO) of the United Nations estimates that about 25% of world food crops are contaminated with mycotoxins [10]. Cases of food destruction owing to high mycotoxin levels, leading to losses of millions of dollars have been reported [11,12]. Wu reported an estimated annual economic loss of between 1-46 million US dollars as a result of fumonisin contamination in animal feed leading to market and animal life losses in the United States [13]. Losses resulting from all mycotoxin-related issues in agriculture in the United States have been estimated to be as high as 1.4 billion dollars annually [14]. Although there are no existing data regarding economic losses caused by mycotoxins in Europe, in 2015, the Rapid Alert System for Food and Feed (RASFF) reported 475 notifications on mycotoxin exposure in food [15]. In addition, Fanelli and Logrieco [16] reported an estimated direct and indirect loss of 100 million Euro due to mycotoxin-wheat epidemic in Hungary in 1998.

The emergence and occurrence of new *Fusarium* metabolites in food crops and products is of great concern. The occurrences of emerging mycotoxins produced by *Fusarium* spp., such as fusaproliferin, beauvericin, enniatins, and moniliformin have been reported in food crops representing an important problem in some parts of the world [17,18]. The risk of human and animal exposure to these mycotoxins has led to continuing elucidation of chemical structures and possible further alteration of *Fusarium* toxin's structure in crops and food products. Gareis et al. [19] observed some cases of mycotoxicosis in animals which did not correlate with the corresponding low-mycotoxin-contaminated feeds they were consuming. The elevated toxicity was ascribed to undetected conjugated forms of mycotoxins that were possibly hydrolysed into their free toxins in the digestive tract of the animals. This is supported by the recent *in vitro* study of Gratz et al. [20] and Ajandouz et al. [21] which revealed the potential hydrolyses of conjugated mycotoxins into free mycotoxins by the microbiota in the human gut. These undetected conjugated mycotoxins, referred to as modified mycotoxins, may be matrix-associated; biologically modified by plants, animals or fungi; or chemically modified by thermal or non-thermal processing [22]. Recently, much attention has been channelled to modified mycotoxins, especially in the developed countries. Several studies have proven the existence of modified mycotoxins in crops and food products [23–34]. Conversely, the limited existence of toxicological data on modified mycotoxins has contributed to the difficulty in ascertaining their toxicity effects and hampered the establishment of overall regulatory limits of *Fusarium* mycotoxins by regulatory bodies to protect

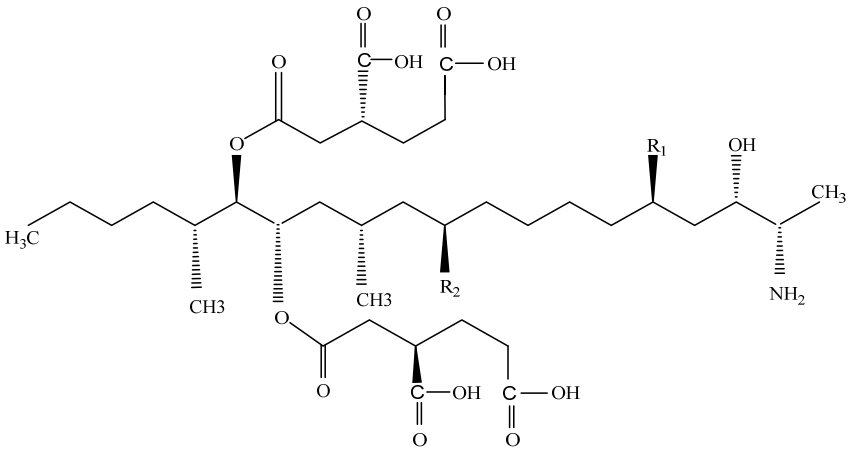
consumer's health. The major *Fusarium* mycotoxins including fumonisins, zearalenone, trichothecenes as well as emerging and modified mycotoxins are briefly discussed hereunder.

1.2 *Fusarium* mycotoxins: origin, chemistry, and distribution

Occurrence of *Fusarium* toxins in agricultural and processed food products is of great concern because of their toxic health effects in humans and animals. Global occurrence data on *Fusarium* mycotoxins have been extensively reviewed [35–37], especially on the major mycotoxins (fumonisins, zearalenone, and trichothecenes) and their possible health effects [38,39].

1.2.1 Fumonisins

Fumonisins (FBs) were first described in South Africa by Bezuidenhout et al. [40]. About 28 FBs analogues have been characterised, and are classified into 4 main groups (A, B, C, and P series), with those belonging to the B series (FB_1, FB_2, and FB_3) being the most abundant natural contaminants of food commodities and of toxicological importance. Each FBs in the B-series has a linear 20-carbon backbone with methyl, hydroxyl, and tricarboxylic acid moieties at various positions along the backbone. FB_1 is the diester of propane-1,2,3-tricarboxylic acid and a pentahydroxyeicosane in which the C-14 and C-15 hydroxy groups are esterified with the terminal carboxy group of propane-1,2,3-tricarboxylic acid (TCA), while FB_2 is the C-10-deoxy analogue of FB_1, FB_3 is the C-5-deoxy analogue of FB_1 (Figure 1.1) [41].



FBs	R_1	R_2
FB_1	OH	OH
FB_2	OH	H
FB_3	H	OH
FB_4	H	H

FBs = fumonisins,
FB_1, B_2, B_3 = fumonisin B_1, B_2, B_3

Figure 1.1. Chemical structure of fumonisins.

FBs compounds are produced by a large array of *Fusarium* species, majorly *F. verticillioides* (formally named *F. moniliforme*) and *F. proliferatum* and other related fungi species including *F. nygamai, F. napiforme*, and *F. Dlamini* [42]. Furthermore, the production of FBs by *Alternaria alternate* f. sp. *Lycopersici* and *Aspergillus niger* has been reported [43,44].

Ingestion of FBs has been associated with several human and animal ailments worldwide including hepatotoxicity, nephrotoxicity, neurotoxicity, immune stimulation, immune suppression, causing several developmental abnormalities, and liver and kidney malfunctions [45–48]. The mechanism of action of FBs is associated with their structural resemblance of sphingosine, sphinganine, and related complex sphingolipids, thus inhibiting sphingosine (sphinganine) N-acetyltransferase (ceramide synthetase) and disrupting the synthesis of sphingolipids [8]. Evidence of accumulation of sphingonine and sphingosine and depletion of sphingolipids by FBs in a variety of cultured cells has been reported [41,42]. Human epidemiological studies have revealed a possible link of the consumption of FBs-contaminated maize (corn) with oesophageal cancer in South Africa, China, North-Eastern Italy, and the south-east of the United States [45,49–51]. Thus, the International Agency for Research on Cancer (IARC) has classified FBs as a group 2B carcinogen (possibly carcinogenic) to humans [52]. Cases of human abdominal pains and diarrhoea were also reported in India, resulting from consumption of mouldy maize or sorghum containing high levels of FBs [53]. Simultaneously, there is an assumption on a possible increase in the risk of neural tube defects because of the human maternal exposure to FBs during the early stages of pregnancy [54]. Recently, the IARC reported the possible association between FBs and stunting in children (IARC, 2015). Cases of animal diseases of most importance equine leukoencephalomalacia (ELEM) and porcine pulmonary oedema (PPO) which resulted to the death of horses and pigs, respectively, as a result of ingestion of FBs-contaminated feed have been reported [46,56–61].

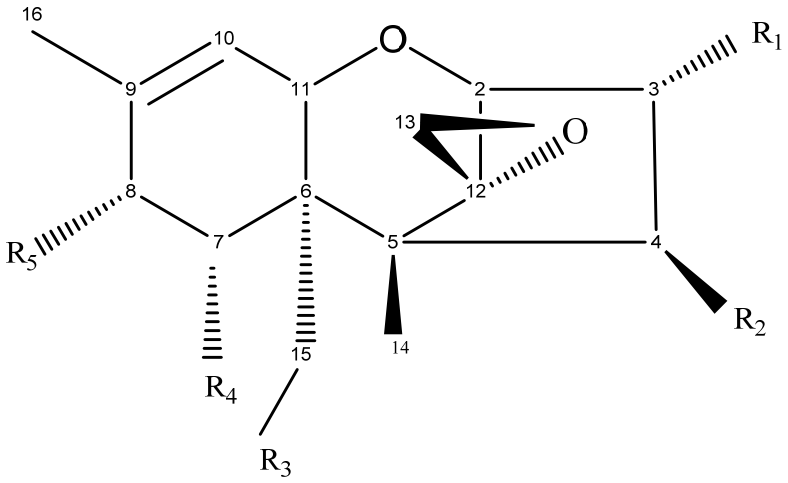
Occurrence of FBs in several cereals, legume crops, spices, and food products all over the world has been established in many studies. The rate of occurrence and levels in food products varies from region to region and is dependent upon several factors including climatic factors, the toxigenic potentials of the fungi as well as other environmental factors (e.g. insect activities). Maize and its products remain the most contaminated because of the susceptibility of the maize crop to FBs-producing fungi. Unsurprisingly, reports on obvious toxicological effects of FBs have always reflected in the regions such as South Africa with high consumption of maize and maize-based products. Unfortunately, processing of cereals into different food forms has contributed little as regards to reducing exposure of consumers. This is apparently because of the thermal stability of FBs. Even when FBs are degraded, they often

form related compounds which may be hydrolysed into the free form in the gastrointestinal tract. More details on the effect of processing on *Fusarium* mycotoxins will be discussed in Section 1.4.

1.2.2 Trichothecenes

Trichothecenes (TH) are a large group of structurally-related sesquiterpenoid mycotoxins produced by a wide range of *Fusarium* spp., although other mould genera such as *Trichoderma, Trichothecium, Stachybotrys, Verticimonosporium, Cephalosporium, Myrothecium*, and *Cylindrocarpon* can also synthesise them [62]. The term TH was derived from the first identified member (trichothecin) of the group. TH have a tetracyclic 12,13-epoxytrichothecene skeleton in common and are divided into 4 categories (type A, B, C, and D) based on their chemical properties which either contains a macrocyclic ester or an ester-ether bridge between C-4 and C-15. Approximately 180 TH exist, but the ones of economic concern include those of type A [T-2 toxin (T-2), HT-2 toxin (HT-2), diacetoxyscirpenol (DAS), and neosolaniol (NEO)] and type B [deoxynivalenol (DON), nivalenol (NIV), and fusarenon X (FUS-X)] because of their frequent occurrence in food commodities and their toxic health effects. Type A and B TH can be distinguished based on their substitution at the C-8. While Type B TH have a keto-group at C-8, Type A TH may have a hydroxyl group (e.g. NEO), an ester group (e.g. T-2), or no oxygen substitution (e.g. trichodermin) at C-8 (Figure 1.2) [63].

Ingestion of TH-contaminated food products has been associated with several human and animal diseases probably because of the epoxide at the $C_{12,13}$ positions, which exhibits toxicological activity [64]. TH show varying degrees of cytotoxic potency based on the type, the dose, and the duration of exposure. They have been revealed as inhibitors of eukaryotic protein synthesis, as well as DNA/RNA synthesis while affecting cell division thus inhibiting mitochondrial function [8,65]. An extensive review on the effects of TH on eukaryotic cells was reported by Rocha et al. [66]. Prelusky et al. [67] and Rotter et al. [68] reported type A TH to be more acutely toxic, while those belonging to type B are implicated in more chronic toxicoses. Of all the TH, clinical data from animal studies suggest that T-2 and DAS are the most potent [8]. In addition to inhibitors of eukaryotic protein synthesis, T-2 and HT-2 induce haematotoxicity, myelotoxicity, growth retardation, and necrotic lesion [69]. At low doses, DON exhibits toxicity often characterised in animals by feed refusal, thus decreasing growth rate. In a higher exposure rate, it expresses immunosuppressive and immunostimulation properties. Epidemiological studies suggest the possibility of DON causing emetic effects in humans [70].



	Trichothecene	R_1	R_2	R_3	R_4	R_5
Type A	T-2	OH	OAc	OAc	H	OIsoval
	HT-2	OH	OH	OAc	H	OIsoval
	DAS	OH	OAc	OAc	H	H
	NEO	OH	OAc	OAc	H	OH
	15-MAS	OH	OH	OAc	H	H
Type B	DON	OH	H	OH	OH	=O
	3-ADON	OAc	H	OH	OH	=O
	15-ADON	OH	H	OAc	OH	=O
	NIV	OH	OH	OH	OH	=O
	FUS-X	OH	OAC	OH	OH	=O

T-2 = T-2 toxin, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, NEO = neosolaniol, 15-MAS = 15-monoacetoxyscirpenol, DON = deoxynivalenol, 3-ADON = 3-acetyl-DON, 15-ADON = 15-acetyl-DON, NIV = nivalenol, FUS-X = fusarenon-X

Figure 1.2. Chemical structure of trichothecenes.

In addition, the study of Razafimanjato et al. [71] revealed the potential of DON decreasing the viability of glial cells responsible for maintaining brain homeostasis, thus causing modifications and possibly participating in the etiology of neurological diseases in which alteration of glial cells are involved. Similarly, NIV has been shown to exert clinical effects such as haematotoxicity and immunotoxicity in mammals. Possible symptoms of TH toxicity include vomiting, headache, dizziness, bleeding, nausea, fever, abdominal distress, dyspnea, weight loss, abortion, and may lead to death. However, the symptoms may vary depending on the TH as well as the animal species. An association of TH (T-2) with alimentary toxic aleukia in humans as a result of consumption of grains contaminated with *F. sporotrichioides* was reported in the Orenburg region in Russia. This led to the death of thousands of people. Similar outbreak of a disease called *akakabi-byo* in Japan, as a result of consumption of *F. graminearum*-contaminated

grains was also reported by Marasas et al. [72]. Other outbreaks of acute poisoning in humans which exhibited symptoms such as vomiting, nausea, diarrhoea, abdominal pain, dizziness, and headache as a result of consumption of *Fusarium*-contaminated grains have also been reported [73]. In addition to the individual toxic health effect exerted by TH toxins, several studies have revealed possible combined effects of these toxins on host animals. Speijer and Speijer [74] observed the antagonistic effect of DON on T-2 in the inhibition of human lymphocytes proliferation. It is noteworthy to mention that ternary combination of type B TH (FUS-X, NIV, and DON) exhibited an antagonistic interaction on the intestinal epithelial cells which is possibly linked to a lower toxicity of FUS-X in the mixture [75]. There exists a potential relationship in the reduction of FUS-X toxicity and the competition between DON and NIV at the substrate binding sites of the de-acetylase, thus leading to a reduced deacetylation of FUS-X [75]. Cases of synergistic interaction exhibited by combination of TH and other mycotoxin such as ZEN and FB_1 have also been reported [76,77]. Harvey et al. [78] and Kubena et al. [79] demonstrated the synergistic and additive effects resulting to growth depression in pigs and broiler chicks, respectively, because of co-occurrence of mixed mycotoxins (DON and FB_1). A synergistic interaction between several combinations of type B TH on epithelial cell toxicity has also been recorded [75,80].

TH are commonly found in agricultural products, especially cereal crops such as wheat, maize, barley, oats, rye, rice, and cereal-based foods worldwide. Natural occurrence of DON in cereals is prevalent, and surveys from South America, Canada, China, and many countries of Europe have shown its frequent occurrence, as well as high levels in cereal crops. The frequent co-occurrence of DON with its acetylated (3-acetyl-deoxynivalenol (3-ADON) and 15-acetyl-deoxynivalenol (15-ADON)) and glycosylated forms (deoxynivalenol-3-glucoside (DON-3G)) (see Section 1.2.4 for more details) have also been recorded. In Europe, type B TH seem to be the most dominant [6], and this has expedited the establishment of regulatory limits for these toxins in various foodstuffs in order to avoid outbreaks of toxicoses [81].

1.2.3 Zearalenone

Zearalenone (ZEN), formally known as F-2 toxin, is a secondary metabolite first isolated, crystallised, and analysed in the 1960s from *Giberella zeae*-contaminated maize. ZEN is a phenolic resorcyclic acid lactone, chemically described as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic-acid-lactone (Figure 1.3) [82]. ZEN is produced by a variety of *Fusarium* fungi species including *F. graminearum, F. culmorum, F. verticillioides, F. cerealis, F. equiseti, F. crookwellense,* and *F. semitectum*. These fungi contaminate crops in the field and under favourable climatic and

environmental conditions, they may produce ZEN prior to harvest. However, production of ZEN during storage has equally been reported by Kuiper-Goodman et al. [83], who observed high level production of ZEN in maize-based feed as a result of improper storage.

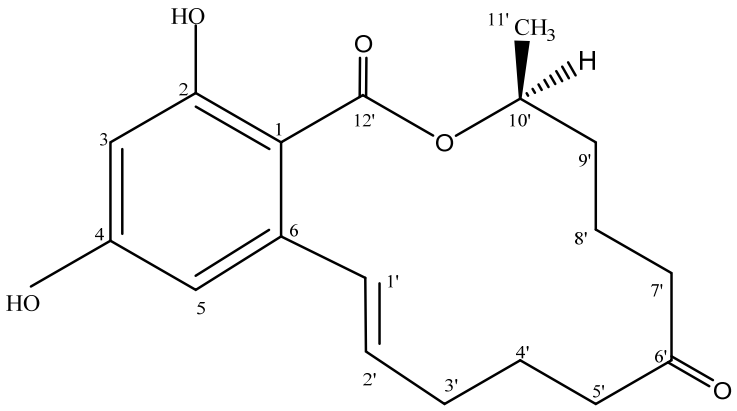


Figure 1.3 Chemical structure of zearalenone.

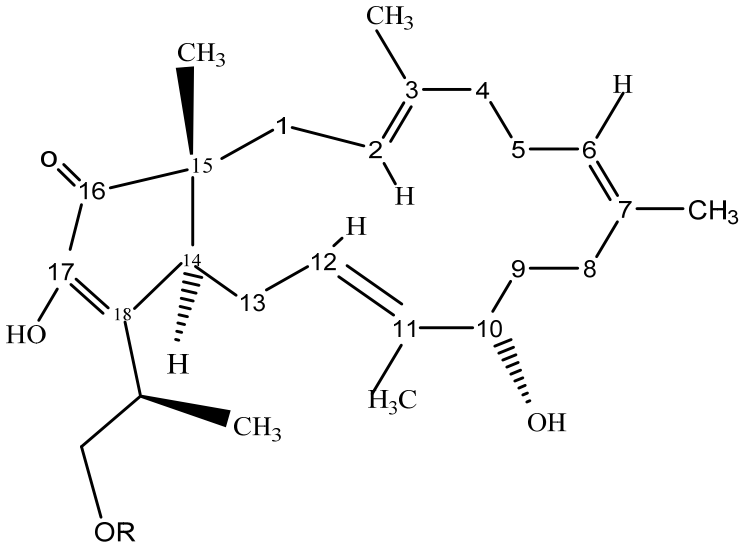
ZEN is commonly found in cereal crops, though its occurrence in other food products such as soybean products, dried fruit and vegetables, and cheese snacks has also been reported [84–86]. ZEN often co-occurs with its derivatives α-zearalenol (α-ZEL) and ß-zearalenol (ß-ZEL) (see Section 1.2.4 for more details), and other mycotoxins including DON, 15-ADON, 3-ADON, NIV, and FUS-X because of the ability of the producing fungi to synthesise more than one mycotoxin which often results to synergistic and/or additive effects on the host organism [9].

ZEN is a non-steroidal oestrogenic mycotoxin, with its structure resembling 17ß-oestradiol, and exerting toxic effects on both animals and humans. ZEN exerts an oestrogenic response because of its high binding affinities for the intracellular oestrogen receptor, and can enhance the proliferation of oestrogen responsive tumour cells [87]. Studies have reported the ability of ZEN to stimulate the growth of oestrogen responsive positive cells, increase uterine weight, modulate the oestrous cycle and compete with oestradiol for oestrogen responsive binding [88]. Its occurrence in foods and feeds has been linked to mammary tumorigenesis and hyperestrogenism, especially in pigs, resulting in adverse effects on the reproductive performance of breeding animals [89]. Although, IARC has classified ZEN as a group 3 carcinogen (not classifiable as to its carcinogenicity to humans), the potential characteristics of ZEN to alter hormonal activity remains an issue of concern. Consequently, studies have shown its genotoxic and/or

carcinogenic effects [90,91]. ZEN has been alleged to cause human cervical cancer and premature initial breast development [92], and an epidemic of precocious pubertal changes in young children in Puerto Rico between 1978 and 1981 [93]. Other authors also reported a possible link between ZEN and the incidence of oesophageal cancer in certain parts of the world in combination with other mycotoxins such as FBs and TH [94,95]. Ingestion of ZEN has exhibited symptoms such as enlargement of mammary glands, vaginal and rectal prolapses, vaginal swelling (vulvovaginitis), testicular atrophy, infertility, prolonged oestrus, reduced sexual drive, stillbirths, abortion, and reduced litter size [67,96].

1.2.4 Emerging and modified *Fusarium* mycotoxins

Studies on *Fusarium* mycotoxins have primarily focused on the occurrence and toxicological effects of FBs, TH, and ZEN on humans and animals as well as prevention and detoxification strategies of these mycotoxins in food chains. However, in recent years, mycotoxin research has broadened upon other *Fusarium* mycotoxins such as fusaproliferin (FUS), beauvericin (BEA), enniatins (ENNs), and moniliformin (MON) often called emerging *Fusarium* mycotoxins. FUS is a bicyclic sesterterpene mycotoxin (Figure 1.4) originally identified from the cultures of *Fusarium proliferatum* [97]. FUS often co-occurs with deacetylated-FUS because of the ability of the producing fungi to synthesise both compounds.

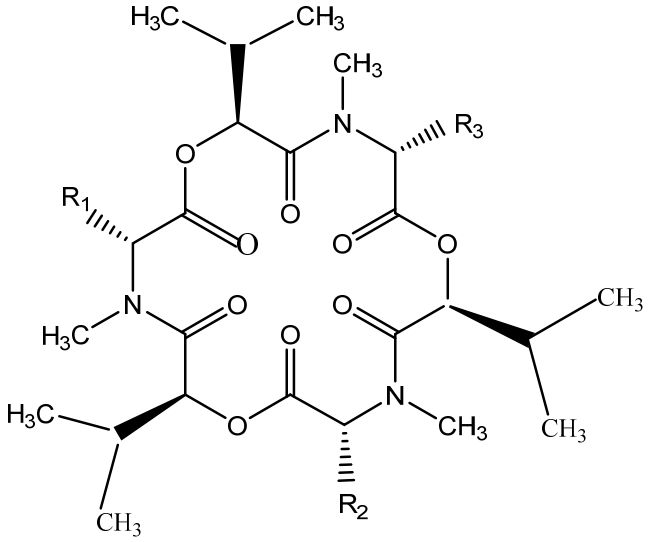


Compound	R
FUS	CH3CO
Deacetyl-FUS	H

FUS = fusaproliferin

Figure 1.4 Chemical structure of fusaproliferin and deacetyl-fusaproliferin

BEA and ENNs were first isolated from cultures of *Beauveria bassiana* and *F. oxysporum* (formally known as *F. orthoceras* Appl. & Wr. Var. Enniatum), respectively. BEA and ENNs are cyclic hexadepsipeptides consisting of alternating D-*α*-hydroxy-isovaleryl- (2-hydroxy-3-methylbutanoic acid) and amino acid units (Figure 1.5), while MON, chemically described as 3-hydroxycyclobut-3-ene-1,2-dione was first isolated from *F. proliferatum* (previously identified as *F. moniliforme*) (Figure 1.6) [97].



Compound	R_1	R_2	R_3
BEA	Phenylmethyl	Phenylmethyl	Phenylmethyl
ENN A	sec-butyl	sec-butyl	sec-butyl
ENN A1	sec-butyl	sec-butyl	iso-propyl
ENN B	iso-propyl	iso-propyl	iso-propyl
ENN B1	iso-propyl	iso-propyl	sec-butyl

BEA = beauvericin, ENN = enniatin

Figure 1.5 Chemical structure of beauvericin and enniatins

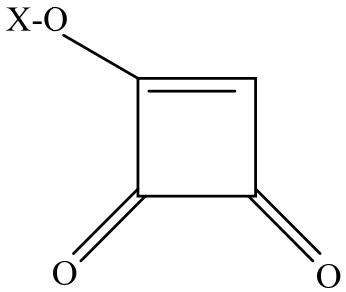
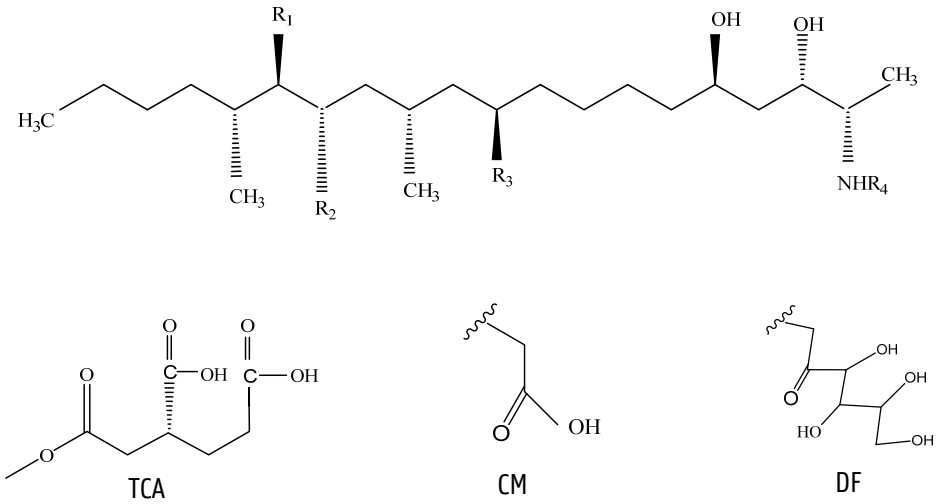


Figure 1.6 The chemical structure of moniliformin. X = H (free acid), Na (sodium salt) or K (potassium salt)

Evidence of occurrence of these toxins (FUS, BEA, ENNs, and MON) in different food products has been reported [18,98–104], thus posing a severe challenge in some parts of the world. Neglecting these *Fusarium* mycotoxins increases the risk of exposure of humans and animals to mycotoxin toxicity because of the possible high incidence

and concentration in food commodities especially cereals and cereal-based products which serve as staple foods in different parts of the world. BEA and ENNs have shown cytotoxic and apoptotic effects on several humans cell lines and animal species [105,106]. They also act as specific inhibitors to cholesterol acyltransferase [107,108]. In addition, ENNs have been found to have a synergistic, additive, and antagonistic toxic effects on Caco-2 cells, because of the possible co-occurrence of ENN analogues [109]. MON is a potent inhibitor of the pyruvate dehydrogenase complex, inducing cardiotoxicity, immunosuppression, muscular weakness, and intestinal problems [110–112]. On the other hand, FUS has exhibited teratogenic and pathogenic effects on human B-lymphocyte cells [113].

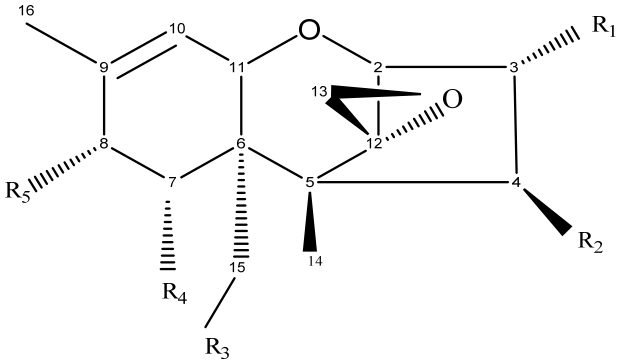
Apart from the emerging *Fusarium* mycotoxins, a recent concern is the occurrence of modified mycotoxins (Figures 1.7 and 1.8). This group of toxins which were originally known as masked mycotoxins, came to limelight in mid-eighties after Gareis et al. [19] reported several mysterious cases of mycotoxicosis that occurred in animals, in which symptoms did not correlate with the low mycotoxin content detected in the corresponding feed. The unexpected high toxicity was attributed to the undetected conjugated forms of mycotoxins contaminating the animal feed.

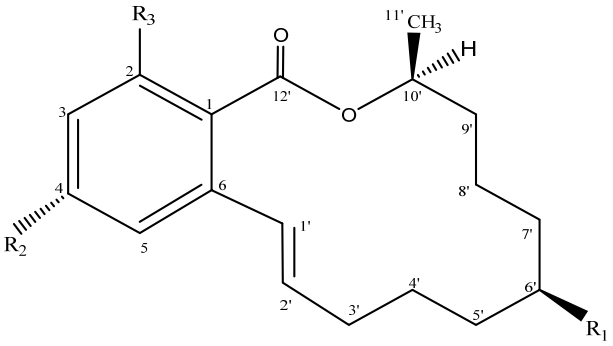


Compound	R_1	R_2	R_3	R_4
Hydrolysed fumonisin B_1	H	H	OH	H
N-(1-deoxy-D-fructos-1-yl)-fumonisin B_1	TCA	TCA	OH	DF
N-(carboxymethyl) fumonisin B_1	TCA	TCA	OH	CM

TCA = tricarballylic acid, CM = carboxymethyl, DF = deoxy-D-fructos-1-yl

Figure 1.7 The chemical structure of modified fumonisins (Hydrolysed fumonisin B_1, *N*-(1-deoxy-D-fructos-1-yl)-fumonisin B_1, *N*-(carboxymethyl) fumonisin B_1)





Compound	R_1	R_2	R_3	R_4	R_5
DON-3G	$OC_6H_{11}O_5$	H	OH	OH	=O
NIV-3G	$OC_6H_{11}O_5$	OH	OH	OH	=O
FUS-X-G	$OC_6H_{11}O_5$	$OCOCH_3$	OH	OH	=O
T-2-3G	$OC_6H_{11}O_5$	$OCOCH_3$	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$
HT-2-3G	$OC_6H_{11}O_5$	OH	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$
HT-2-4G	OH	$OC_6H_{11}O_5$	$OCOCH_3$	H	$OCOCH_2CH(CH_3)_2$

DON-3G=deoxynivalenol-3-glucoside; NIV-3G=nivalenol-3-glucoside; FUS-X=fusarenon-X-glucoside; T-2-3G=T-2-3-glucoside ; HT-2-3G=HT-2-3-glucoside; HT-2-4G=HT-2-4-glucoside

Compound	R_1	R_2	R_3
ZEN-14G	=O	$OC_6H_{11}O_5$	OH
ZEN-14S	=O	OSO_3	OH
ZEN-16G	=O	OH	$OC_6H_{11}O_5$
α-ZEL	OH (α)	OH	OH
α-ZEL-14G	OH (α)	$OC_6H_{11}O_5$	OH
β-ZEL	OH (β)	OH	OH
β-ZEL-14G	OH (β)	$OC_6H_{11}O_5$	OH

ZEN-14G=zearalenone-14- glucoside; ZEN-14S=ZEN-14-sulphate; ZEN-16G=ZEN-16- glucoside; α-ZEL=α-zearalenol; α-ZEL-14G= α-ZEL-14-glucoside; β-ZEL=β-zearalenol; β-ZEL-14G= β-zearalenol-14- glucoside

Figure 1.8 The chemical structure of modified trichothecenes and zearalenone

This led the International Life Science Institute (ILSI) to define masked mycotoxins as mycotoxins derivatives which are not detectable by conventional analytical methods because of the alteration in their chemical structures and unavailability of reference standards, leading to an underestimation of the mycotoxin concentration [114]. Unfortunately, these compounds may be released to their free forms in the gastrointestinal tract of the animals exerting their toxic effects. Because of the ambiguous use of “masked” along with other terms such as “bound”, “hidden”, and “conjugated”, Berthiller et al. [114] suggested the description of masked mycotoxins to be used for solely conjugated mycotoxins generated by plant metabolites. However, since there are other structural altered mycotoxins excluding plants conjugates, Rychlik et al. [22] proposed a systematic definition consisting of four

hierarchic levels to include all potential relevant forms in which mycotoxins and their modification can occur (Table 1.1).

The first level differentiates the free mycotoxins from those being matrix-associated and those in which their chemical structures have been biologically or chemically modified by plant, animal or fungus metabolism, or during food processing (thermally or non-thermally forms). In view of the definition, it is noteworthy to highlight that some of these compounds may belong to more than one category [22]. The acetylated DON (3-ADON and 15-ADON) classified as free mycotoxins may also be classified as modified mycotoxins. In an attempt to detoxify DON, plants were genetically transformed by inserting a 3-O-acetyltransferase, thus allowing the acetylation of DON to 3-ADON, a trait which plants do not possess naturally. This opened the frontiers of discourse towards classifying 3-ADON under biologically modified mycotoxins [115,116].

Table 1.1 Systematic definition of modified mycotoxins by Rychlik et al. [22]

1st level	2nd level	3rd level	4th level	Example
Free mycotoxins				DON, 3-ADON, 15-ADON
Matrix-associated mycotoxins	Complexes, physically dissolved or trapped covalently bound			FBs bound to starch, DONoligosaccharides
Modified mycotoxins	Biologically modified	Functionalised (phase 1- metabolites)		AFB_1-epoxide
		Conjugated (phase 2 – metabolites	Conjugated by plants (= masked according to ILSI)	DON-3G
			Conjugated by animals	DON-3/8/15-GLU, HT2-3/4-GLU
			Conjugated by fungi	ZEN-14S
	Chemically modified	Differently modified		DOM
		Thermally formed		norDON A-C, N-carboxymethyl-FB_1
		Non-thermally formed		DON-sulfonate, norDON A-C (under alkaline conditions)

DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, FBs = fumonisins, AFB_1 = aflatoxin B_1, DON-3G = deoxynivalenol-3-glucoside, DON-3/8/15-GLU = deoxynivalenol-3/8/15-glucuronide, HT2-3/4-GLU = HT-2 toxin-3/4-glucuronide, ZEN-14S = zearalenone-14-sulphate, DOM = de-epoxy deoxynivalenol.

The occurrence of structural altered mycotoxins in cereals is mainly attributed to phase I metabolism, in which xenobiotics are subjected to hydrolysis, or phase II metabolism which deactivates phase I activated metabolites or xenobiotics through conjugation with endogenous hydrophilic molecules such a glucose, sulphate or amino acid moieties [117]. Among the modified mycotoxins, TH and ZEN metabolites especially the glucosides have received more attention with reports showing their natural occurrence in cereals (maize, wheat, oat, barley, sorghum) as well as cereal-based products including bread, beer, and breakfast cereals [26,34,109–111]. In addition, natural occurrence of modified forms of other TH such as T-2, HT-2, FUS-X, NIV, DAS, and NEO have equally been reported [27,112,113,118,119]. Occurrence of modified FBs have also been reported in cereals [114–116], although the masking phenomenon of these mycotoxins involves the covalent bond formation between the tricarboxylic moiety and hydroxyl groups of carbohydrates or the amino groups of amino acids upon heating, or the non-covalent binding through physical entrapment of the mycotoxins to matrix constituents [106]. Cases of occurrence of modified FBs in processed food products such as gluten-free food products [117], corn flakes [26], and heat-processed corn foods [120] have been reported.

Though toxicological data are still limited, the occurrence of modified mycotoxins is extrapolated to add substantially to the overall mycotoxins levels and toxicity. The increase of toxic health effects by modified mycotoxins may be either direct or indirect via hydrolysis, or release from the matrix during digestion into the free compounds [121]. Comparative cytotoxic effects of DON and its acetylated derivatives on a non-transformed intestinal epithelial cell line using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) revealed that 3-ADON exerted less toxic effects (EC_{80} value of 125 µM) when compared to the free toxin (EC_{80} value of 16.5 µM), while the reverse was the case with 15-ADON (EC_{80} value of 10.5 µM) [80]. Their findings were in agreement with the previous studies of Pinton et al. [120] and Kadota et al. [122], who compared the toxicity of DON, 3-ADON, and 15-ADON on porcine intestinal epithelial cells and human intestinal Caco-2 cell, respectively. Pinton et al. [120] reported a reduction in cell proliferation by DON and its acetylated derivatives in the ranking order of 3-ADON (13%) < DON (60%) < 15-ADON (69%), while Kadota et al. [122] observed the same trend on the interleukin-8 production in Caco-2 cell. Further *ex vivo* (porcine intestinal explants) and *in vivo* (jejunum from piglets) analysis showed that 15-ADON exerted more toxicity than DON and 3-ADON [120]. A much earlier study by Forsell et al. [123] buttressed this view when mice were exposed to acute oral doses of DON and 15-ADON. DON and its metabolites are able to increase the permeability of the intestinal epithelial layer by decreasing the expression of tight junction proteins [124]. This can be worsened by a reduction in cell proliferation thus increasing susceptibility to pathogens. While the

consistency of results suggests a trend, these protocols need to be replicated in different animal models while minimizing variations within experimental units.

The co-contamination of DON with other mycotoxins and metabolites of DON, and their potential synergistic and additive effects remains a knowledge gap. Eriksen et al. [125] compared DON, its acetylated and deepoxy metabolites on Swiss mouse 3T3 fibroblasts. Their findings showed a similar lower toxic effect by 3-ADON, whereas DON and 15-ADON had equal effects. De-epoxy deoxynivalenol (DOM) was 50 times less toxic than DON [125]. The reduced toxic effect of DOM is attributed to the de-epoxidation of the 12, 13-epoxy ring in the structure of TH, which is the essential functional group alleged to cause toxicity [126]. Regarding the glucosylated form of DON, Poppenberger et al. [127] reported the reduced ability of deoxynivalenol-3-glucoside (DON-3G) to inhibit protein synthesis of wheat ribosomes *in vitro* compared to DON. Pierron et al. [128] studied the possible toxic effect of DON-3G in comparison with DON on the intestine using the human intestinal Caco-2 cell line and porcine jejunal explants. Their investigation revealed the inability of DON-3G to bind to the ribosome thus decreasing its intestinal toxicity when compared to DON. This is in line with the *in vitro* cytotoxicity study of DON-3G on porcine intestinal epithelial cells ranking DON-3G as the least toxic compared to DON and its acetylated forms [129]. In addition, an *in vivo* study demonstrated that DON-3G has a low absolute oral bioavailability in broiler chickens compared to DON [130]. DON-3G is not hydrolysed to DON in broiler chicken similar to the trend reported in different *in vitro* studies [131,132]. In contrast with the study on broiler chicken, Broekaert et al. [130] observed that when pigs were orally administered with DON-3G, they demonstrated a complete hydrolysis of DON-3G to DON. It is noteworthy to mention that the absorbed fraction was approximately 5 times lower than after oral administration of DON. While *in vitro* studies of DON-3G suggest less toxic effects [131], the latter study on pig proves that the toxicological significance of DON-3G should not be neglected especially across different animal species. Apart from the ability of modified metabolites of DON to exert direct toxic effect on animal or human host, a major concern is their hydrolyses into their free forms after ingestion. Studies have reported the possible potentials of these metabolites being hydrolysed to their free forms [20,21]. In order to understand the transformation of 3-ADON and 15-ADON to DON, Ajandouz et al. [21] studied the deacetylation activity of 3-ADON and 15-ADON by enzymes, bacteria, cells, and tissues present in humans. Interestingly, they observed that 3-ADON was more prone to deacetylation than 15-ADON, while small intestine and liver are the major sites of deacetylation of 3-ADON and 15-ADON in humans.

The toxicity of 4-acetyl NIV (FUS X) on Swiss mouse 3T3 fibroblasts showed that 4-acetyl NIV exhibited 1.7 times more toxic effects than NIV [125]. This trend was also observed in previous studies by Visconti et al. [133], and Eriksen

and Alexander [134]. These findings were in line with the study of de-epoxy T-2 using the rat skin irritation assay. Their result showed that de-epoxy T-2 exhibited a 400 times less toxic effect than the corresponding T-2 [135]. Similarly, the cytotoxicity effects of ZEN and its major metabolites α-ZEL and β-ZEL on cultured human Caco-2 cells revealed variable toxic effects of ZEN and its metabolites with observation showing that the toxic effects seem to be relieved by the metabolism of ZEN into α-ZEL and β-ZEL [136]. Othmen et al. [137] reported that α-ZEL and β-ZEL inhibited cell viability, protein and DNA syntheses, and induced oxidative damage, and over-expression of stress proteins. However, α-ZEL and β-ZEL exhibited lesser toxicity than ZEN, with β-ZEL being the more active of the two metabolites. A reverse toxicity trend was observed in the oestrogenic potential of these compounds [138], with α-ZEL being ranked as the most toxic, followed by ZEN, and then β-ZEL. This trend of toxic effect was shown by Ayed et al. [139]. Zearalenone-14-sulphate (ZEN-14S) and zearalenone-14-glucoside (ZEN-14G) exhibited low oestrogenic potential which is attributed to their inability to bind to the oestrogen receptor [140,141]. Apart from the low oestrogenic potential of ZEN-14G, *in vitro* study also showed a lower toxicity of ZEN-14G in respect to its free form (ZEN). Dellafiora et al. [142] studied the hydrolysis of ZEN-14G to its free form (ZEN) in the bovine blood and blood components including plasma, serum, and serum albumin. Their study revealed the reduction in ZEN-14G in all the treatments thus leading to the release of ZEN with a significant amount of zearalenol isomers (α-ZEL and β-ZEL) in whole blood. The trend observed by these authors corresponded with the earlier *in vivo* studies by Gareis et al. [19] and Versilovskis et al. [143] which demonstrated the complete hydrolysis of ZEN-14G to its aglycone ZEN during digestion in pigs and rats, respectively. In respect of modified FBs, Dall'Asta et al. [144] reported the possible release of hidden FBs under gastrointestinal conditions after application of an *in vitro* digestion model to raw maize, suggesting that gastrointestinal enzymes are able to destroy the matrix-fumonisin interactions. However, Falavigna et al. [145] reported the stability of covalently bound FB_1 conjugates upon *in vitro* digestion, whereas non-covalently bound compounds were hydrolysed to their free forms.

1.3 Regulation of *Fusarium* mycotoxins

The aim of setting maximum or recommended acceptable levels for mycotoxins in food and feed commodities is to preserve the human and animal health, since it is virtually impossible to eliminate these arrays of toxins from agricultural products. Mycotoxin regulations are established based on the availability of toxicological and exposure data, and the knowledge of the distribution of mycotoxin concentrations within the commodity [146]. Further, the

availability of reliable analytical methods is a prerequisite for possible enforcement of regulation, while putting into consideration the social-economic factors such as trade interests and food security issues. Worldwide efforts to control mycotoxins have mostly been directed toward controlling aflatoxins because of their toxic effects and their carcinogenic potentials. In this regards, about a hundred countries have developed specific limits for mycotoxins in food and feed, with the population in these countries representing 87% of the world's inhabitants [147]. However, the maximum levels differ widely on national levels base on what a nation constitutes a safe maximum level for humans.

The recent harmonisation of mycotoxin regulations by several economic communities (eg European Union(EU), Mercado Cómun del Sur (MERCOSUR), Australia and New Zealand, and Association of Southeast Asian Nations (ASEAN)) have overruled the existing national regulations [146]. This complements the Codex Alimentarius Commission (CAC) international standards for different mycotoxins in foods and feeds most especially as relates to international trade [148]. While there are regulations for *Fusarium* mycotoxins in these economic communities, it is important to mention that regions such as sub-Saharan Africa (SSA) still lack such potentials because of political and socio-economic challenges. Countries within SSA depend on regulations set by other regions. The establishment of maximum limits of these mycotoxins is based on the scientific opinion of the European Food Safety Authority (EFSA) and Food and Agricultural Organisation (FAO)/World Health Organisation (WHO) Joint Expert Committee on Food Additives of the United Nations (JECFA). Mycotoxins with established regulations include those produced by *Fusarium* fungi. In this regard, the EU has implemented the most comprehensive regulations in the world for *Fusarium* mycotoxins (FBs, DON, and ZEN) in foodstuffs to protect consumers, thus have been published in Commission Regulation (EC) No 1881/2006 (Table 1.2). Although, there are no maximum limits set for T-2 and HT-2 by the EU, an indicative level of T-2 and HT-2 in cereals and cereal-based products was introduced according to the Commission Recommendation (No 2013/165) [149]. Furthermore, the EFSA has amalgamated opinions on mycotoxin risk assessments, thus calculating a tolerable daily intake (TDI) for FBs at 2 µg/kg body weight (b.w.) per day, 1 µg/kg b.w. per day for DON, 0.25 µg/kg b.w. per day for ZEN [150], and 0.10 µg/kg b.w. per day for the sum of T-2 and HT-2 [150,151].

Table 1.2 European Union regulatory limits (No 1881/2006) and indicative levels (No 2013/165) for Fusarium mycotoxins in foodstuffs

Mycotoxin	Foodstuffs: maximum levels	µg/kg
Sum of Fumonisin B_1 and FB_2 (maximum levels)	• Unprocessed maize	4,000
	• Maize intended for direct human consumption, maize- based foods for direct human consumption, with the exception of foodstuffs ‡ §	1,000
	• ‡ Maize-based breakfast cereals and maize-based snacks	800
	• § Processed maize-based foods and baby foods for infants and young children	200
Deoxynivalenol (maximum levels)	• Unprocessed cereals other than durum wheat, oats and maize	1,250
	• Unprocessed durum wheat and oats	1,750
	• Unprocessed maize	1,750
	• Cereals intended for direct human consumption, cereal flour (including maize flour, maize meal and maize grits), bran as end product marketed for direct human consumption and germ, with the exception of foodstuffs listed in †	750
	• Pasta (dry)	750
	• Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals	500
	• † Processed cereal-based foods and baby foods for infants and young children	200
Zearalenone (maximum levels)	• Unprocessed cereals other than maize	100
	• Unprocessed maize	200
	• Cereals intended for direct human consumption, cereal flour, bran as product marketed for direct human consumption and germ, with the exception of foodstuffs listed in †, ‡, §	75
	• † Maize intended for direct human consumption, maize flour, maize meal, maize grits, maize germ and refined maize oil	200
	• Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize snacks and maize based breakfast cereals	50
	• Maize snacks and maize based breakfast cereals	50
	• ‡ Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children	20
	• § Processed maize-based foods for infants and young children	20
Sum of T-2 and HT-2 (indicative values)	Unprocessed cereals	
	• Barley (including malt) and maize	200
	• Oats (not shelled)	1,000
	• Wheat, rye and other cereals	100
	Cereals for direct human consumption	
	• Oats	200
	• Maize	100
	• Other cereals	50
	Cereal products for human consumption	
	• Oats bran and oats flakes	200
	• Bran of cereals with the exception of oats bran and oats flakes, and milling products of maize	100
	• Other milling products of cereals	50
	• Breakfast cereals with the inclusion of formed cereal flakes	75
	• Bread (with the inclusion of small bakery products), pastry, cookies, cereal snacks and pasta	25
	• Processed cereal-based foods for infants and young children	15

While there are evidences of the occurrence of modified mycotoxins in food and feed products, it is presently impossible to establish regulations that protect consumers because of lack of sufficient exposure and toxicological data. An assessment of the co-occurrence of these modified forms of mycotoxins in foods and feeds, and an estimation of the human and animal dietary exposure as compared with the free mycotoxins remains a missing link. Till date, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) assumed an equal toxicity of all modified forms as their free toxins [150,152]. Studies on this subject have been fragmented and hence unable to make quantum leaps in filling the voids of unanswered questions.

Literature data shows that modified forms of mycotoxins may add substantially to the overall mycotoxin levels, in particular for DON, ZEN, and FBs (Section 1.2.4), thus, the Joint FAO/WHO Expert Committee has considered DON metabolites (DON-3G, 3-ADON, and 15-ADON) as an additional contributing factor for dietary exposure to DON [153]. The EFSA CONTAM Panel notes that there is a need for more information on the chemical structures of modified mycotoxins and for further work to identify modified mycotoxins not yet characterised. The nomenclature, and abbreviations need some sort of standardization in respect to mycotoxins and their modified forms. The panel proposed validation and increased sensitivity in routine analytical methods.

The fate of modified forms upon food and feed processing needs further investigation. This necessitates an urgent need for more research on the occurrence and the potential health effect of modified mycotoxins, as well as understanding the behaviour of modified mycotoxins during food processing. In this regard, standardization of experimental protocols and clinical testing across laboratory and regions is critical and timely. More research efforts should be geared toward development of reference standards for modified mycotoxins. This will offer a platform for easy detection and quantification of modified mycotoxins in food and food products across the globe.

1.4 *Fusarium* mycotoxins in sub-Saharan Africa

Mycotoxin contamination remains one of the major food safety concerns in the developing world especially in the SSA. In recent years, efforts and attention have been channelled toward surveillance and control of these toxins, especially the aflatoxins in food and feed systems. This is evident in the establishment of an innovative consortium with the acronym PACA (Partnership for Aflatoxin Control in Africa) with the aim to coordinate aflatoxin mitigation and management across the agriculture, health, and trade sectors, while safeguarding consumer's health and

facilitating trade. Second, the use of a biocontrol product (aflasafe™) which contains atoxigenic strains of *Aspergillus flavus* to reduce mycotoxins specifically aflatoxins have been encouraged in SSA. Application of Aflasafe came to limelight following the breakthrough technology developed by the International Institute of Tropical Agriculture (IITA) in collaboration with the United States Department of Agriculture, Agricultural Research Service (USDA-ARS). In addition, it is also important to mention that there exist aflatoxins regulation in some countries of SSA to protect consumers from the toxigenic effects caused by these toxins. Notwithstanding the efforts toward aflatoxin control in the regions, the menace caused by the contamination of mycotoxins in food and feed products in SSA keeps occurring, probably because of the food insufficiency problem faced by the region. In 2016, the most recent outbreak of mycotoxicosis was reported in Tanzania which led to about 14 deaths as a result of consumption of aflatoxin-contaminated cereals [154], thus suggesting the need for a more integrated approach toward mycotoxin control. Other major health issues in humans and animals as well as economic problems caused by mycotoxins in SSA have not been expansively documented.

Mycotoxin contamination in SSA is influenced by several factors such as the environmental conditions, social economics, and the food production system. Environmental conditions especially high humidity and temperatures in SSA favour fungal proliferation resulting in contamination of food and feed [155]. The recent trend in climatic change further complicates the already precarious situation coupled with the parallel focus of SSA to only *Aspergillus*-produced mycotoxins (aflatoxins), thus neglecting other mycotoxins such as the *Fusarium* mycotoxins. Although *Fusarium* mycotoxins have been associated with temperate climate, Magan et al. [156], and Paterson and Lima [157] emphasised on the importance of climate in fungal colonisation, as well as mycotoxins contamination of foods and food products. Unfortunately, SSA has been reported as a region of higher vulnerability to the impact of global climate change because of its sole dependence on the weather and climate variables for agricultural production [158]. SSA is envisaged to have an increase of 5% to 8% of arid and semi-arid regions which perhaps will cause an increase in drought, and thus may lead to increased crop stress and possibly mycotoxins contamination [159]. This factor may have exposed SSA to *Fusarium* mycotoxin contamination. Tirado et al. [160] highlighted the correlation between FBs occurrence and drought stress, as observed in maize planted during the dry season in south and eastern Africa. The trend of an increase in FBs production in dry weather was also reported by Munkvold and Desjardins [161].

Another possible route of exposure to *Fusarium* mycotoxins in SSA is through trade. Fungi can easily spread from one area to another, and considering that there are no strict regulations and control systems concerning mycotoxins

in this region, SSA is often exposed to contaminated foods and products through global trade. Contrary to the state of research on *Fusarium* mycotoxins in Europe and other parts of the world, few studies have investigated the occurrence of these secondary metabolites in food and feed commodities, with the available data showing a high incidence of *Fusarium* mycotoxins in crops and food products in SSA [162–167]. A Biomin survey on the global mycotoxin threat also reported high incidences of ZEN (91%) and FBs (88%) in the majority of samples from Africa [168]. The data available on the occurrence of *Fusarium* mycotoxins in SSA since the year 2000 was recently reviewed by Chilaka et al. [169], with levels as high as 45,450 µg/kg for FB_1 [170], 3,842 µg/kg for DON [167], 14,900 µg/kg for ZEN [165], 1,923 µg/kg for MON [170], 1,703 µg/kg for 3-ADON [171], 2,056 µg/kg for FUS [172], and 1,020 µg/kg for fusaric acid [172] in food commodities. Despite the increasing concern on *Fusarium* mycotoxins and their modified forms worldwide, SSA has placed little importance on the occurrence and detection of these mycotoxins in crops and food products, as well as their possible deleterious effects. Perhaps this could be as a result of the non-availability of analytical facilities and the prevalence of food insecurity in the region, which are some of the factors affecting the promotion of mycotoxin regulations (Section 1.3) [146]. Another school of thought as highlighted earlier is probably due to the special attention accorded to only aflatoxins in the region, thereby neglecting other toxins.

Although regulation exist for aflatoxins (Section 1.3), there is an absence of regulations governing the control of *Fusarium* mycotoxins in SSA. Thus, subjecting the region to strictly depend on maximum levels in regulations and guidelines of the EU and the CAC on *Fusarium* mycotoxins without putting into considerations the feeding habits, food security status, occurrence levels in the region, and the genetic/hereditary dispositions (genetic and environmental interactions) of the people that make up the region. The absence of regulation is thus attributed to the lack of sufficient scientific data (occurrence, exposure, and toxicological) and the socio-economic factors, such as public ignorance, hunger (as well as hidden hunger), as well as political and economic instability. It is noteworthy to mention that only a few of the countries in SSA have food control administrative systems (e.g. National Agency for Food and Drug Administration and Control (NAFDAC) Nigeria, South African Bureau of Standards (SABS), Kenya Bureau of Standards (KEBS)) that are functional. In most cases the weak regulatory bodies are led (most often by political appointees) most times by personnel and stakeholders with minimal background knowledge about food toxins.

1.5 Mitigation strategies of *Fusarium* mycotoxins during processing

Over the years, the scientific community has proposed good agricultural practices (GAP), followed by implementation of good manufacturing practices (GMP), and hazard analysis and critical control points (HACCP) during food processing as a strategic measure in addressing the problems posed by fungi and mycotoxins in the food system. Food processing may be physical (cleaning and milling processes, physical adsorption, and thermal processes), chemical (use of ammonia, calcium hydroxide, and sulphur containing compounds) or biological (malting, brewing, and fermentation). The degree of reduction of mycotoxin concentrations in food crops and feeds by processing is dependent on the matrix type, the mycotoxin, as well as the processing method, and different conditions employed. Besides studying the effect of processing on mycotoxins, it is important to place in mind the possibility of free mycotoxins co-occurring with their modified forms, or the free mycotoxins being modified and fragmented into other forms during food processing which may not be easily detected by routine methods. Lack of awareness of these mitigation processes has limited SSA to progressively reduce mycotoxins in foods and feeds. Creating awareness on the effect of implementation of GAP, GMP, and HACCP in the control of toxic metabolites in the food system will be ideal to some extent in reducing the risk of mycotoxin exposure in both the rural and urban communities in SSA.

1.5.1 Cleaning and milling

Cleaning and sorting are considered to be the first step of physical decontamination. These techniques are regarded as superior methods because they pose no risk of producing degradable products which subsequently may be toxic [173]. These methods are dated back as old as the beginning of mankind. Several studies have reported the efficiency of physical decontamination methods such as sorting, washing, dehulling, and removal of visible mouldy and floating kernels in the reduction of different types of mycotoxins in foods irrespective of the grain type [174–181]. Reduction between 26% and 69% of total FBs in maize was observed by Sydenham et al. [178] as a result of cleaning, prior to further processing. A 32% reduction of FBs levels in maize in an industrial mill was also reported by Scudamore and Patel [182]. The same trend was observed by van der Westhuizen et al. [181], who recorded a reduction range of 27%-93% of FBs after sorting contaminated maize. Furthermore, Pascale et al. [177] and Scudamore and Patel [183] observed reduction of T-2 (62%), HT-2 (53%), and DON (50%) in wheat grains after cleaning. The reduction recorded by these authors may be ascribed to the fact that mycotoxins are often concentrated in dust and broken kernels because of their susceptibility to fungal infection and subsequent

mycotoxin production. Thus, the percentage of mycotoxin reduction by cleaning and sorting of grains is determined by the physical condition of the grains, as well as the type and effectiveness of the cleaning method. In addition, milling plays a potential role in the reduction of *Fusarium* mycotoxins in grains. However, the problem often encountered is the differential toxicity of the fractions resulting from grain separation. Lee et al. [184], Dexter et al. [185], and Lancova et al. [186] registered the reduction of DON during milling of wheat. This was in agreement with the study of Tibola et al. [187], who reported a higher deposition of *Fusarium* mycotoxins in wheat bran after milling. A similar trend was observed in respect to emerging *Fusarium* mycotoxins. Reduction of 71% and 79% of ENN B and ENN B_1 in wheat flour, respectively was recorded by Vaclavikova et al. [188] as a result of milling, with the highest concentrations of ENN B and ENN B_1 being detected in the bran and shorts. Moreover, similar results were also reported regarding distribution of modified mycotoxins in cereals after milling [187,189,190]. The study on the fractionation of DON and DON-3G in milling fractions showed a similar trend with white flours containing approximately 60% of the content in unprocessed wheat grains [189]. The reduction reported by these authors is attributed to the fact that during dry milling, the highest amounts of mycotoxins are concentrated in the fractions of the commodity (bran) that are less likely to be used for food production, though these higher contaminated fractions mostly end up as animal feed. Furthermore, wet milling of maize has shown to result in the reduction of mycotoxins. Mycotoxins may dissolve into the steep water or be distributed among the by-products, while the starch remains relatively free from mycotoxins [191–193].

1.5.2 Thermal treatment

Several other methods such as thermal treatment used in food processing have been studied to understand its effects on mycotoxins. Mycotoxins are generally heat stable and as such are not easily destroyed during most normal cooking processes [194,195]. However, at very high temperatures, reduction has been reported to occur although this may be as a result of reactions resulting in the formation of products with altered chemical structures. Ryu et al. [196] proved the effectiveness of thermal treatment (extrusion cooking) on the reduction between 66%–83% of ZEN at temperature ranging from 120 °C-160 °C. Scott and Lawrence [197] also reported 60%-100% reduction of FBs when heating dry and moist corn meal at 190 °C (60 min) and 220 °C (25 min), respectively. In addition, Shephard et al. [198] in their study using a traditional South African method for production of maize porridge, observed about 23% reduction in FBs concentration. Notwithstanding the FBs reduction reported during thermal processing, it is important to state the frequent occurrence of bound FBs in thermally treated foods because of the binding of FBs

with matrix constituents through covalent interaction at high temperatures via a Maillard-type reaction [199]. This is evidenced in the studies available on the effect of thermal treatment on FBs, which indicated that the largest reduction of FBs occurred at a temperature of 160 °C or more in the presence of glucose [200]. The main products were *N*-carboxymethyl FBs and *N*-deoxyfructosyl FBs although upon alkali treatment, a hydrolysed form may be formed by the cleavage of both carballylic moieties [200]. These bound FBs are not detectable by the basic routine analytical methods, thus may explain the reduction reported.

In the case of TH such as DON, there have been lots of contradicting reports by different authors on the effect of thermal processing. While Bergamini et al. [201], Kostelanska et al. [189], Numanoglu et al. [202], and Vidal et al. [203] reported a reduction of DON content in bread; Lancova et al. [186] and Scudamore et al. [204] recorded no effect in DON concentration by thermal processes. This conflicting disparity may be attributed to varying baking temperatures, baking procedures, and ingredients used. Furthermore, the analytical methods used and experimental conditions may have contributed to the variation in the trends observed by these scientists. Interestingly, De Angelis et al. [205] documented an approximately 18% higher level of DON in bread when compared to the original flour, which is in line with the earlier study by Young et al. [206]. The increase may be explained by the release of DON from their modified forms of DON-3G. This phenomenon corresponded with the significant drop of DON-3G levels in the bread, and may be due to the activities of yeast during fermentation. In contrast, Vidal et al. [203] reported an increase in DON-3G during baking. Moreover, the same authors investigated the effect of bread baking on T-2, HT-2, and their glucoside conjugates and observed a reduction of the concentration of T-2 (range: 63% to 74%) in bread as compared with the original flour, while HT-2 levels appeared to be less affected [205]. Reduction of T-2 may be ascribed to the partial conversion of T-2 to HT-2 during yeast fermentation operated by the carboxylesterase naturally present in cereal-based products and/or partial degradation of T-2 due to thermal treatment. In the case of T-2 glucoside (T-2G) and HT-2 glucoside (HT-2G), the same trend was recorded in HT-2G whilst a reverse behaviour was found for T-2G. These results agreed with the report of Humpf and Voss on the possible formation of unknown biologically active compounds or the reversible binding of the toxin to sugars or proteins in the food/feed matrix during heat treatment [200].

1.5.3 Fermentation

Another universal biological food processing method is fermentation. In SSA, fermentation is one of the most technologically appropriate methods for food processing because of its affordability and suitability for the

production of staple foods in rural and urban regions. Although fermentation offers many advantages such as food preservation, enhanced sensory qualities, increased nutritional value and variety of food type, reduced anti-nutritional compounds, improved functional properties, and food safety, the living cells and enzymes used during this process may lead to the liberation or transformation of mycotoxins into modified mycotoxins. Furthermore, *Fusarium* fungi, when present during fermentation, are still capable of growing and synthesising mycotoxins. Information on the effect of fermentation on *Fusarium* mycotoxins, especially using the African traditional fermentation methods is limited. So much diversity of results has been reported by different authors on the effect of fermentation on mycotoxins (especially DON) during bread making. While some studies recorded a mean reduction of DON in fermented dough [207–209], others reported stability [210] and increase of DON concentration during fermentation [201,203,206], although the increase observed by Vidal et al. [203] was a combined effect of kneading, fermentation, and proofing. These conflicting reports could be as a result of several factors such as differences in technology, process temperature, and initial concentration of the mycotoxins. Interestingly, the possible explanation of increase of DON concentration as reported by the latter authors may be as a result of the enzymatic release of bound forms of DON occurring in the raw materials. Kostelanka et al. [189] reported an increase of up to 145% of DON-3G in the fermented dough when bakery improver's enzymes (16% of protease, 39% of xylanase) were added. A similar report on the increase of DON (3.5%) after fermentation was observed in wheat germ-enriched bread [211].

A study on the effect of fermentation on mycotoxins during local processing of Nepalese traditional beer using experimentally contaminated maize, showed stability of FB_1 throughout the fermentation process, while a 50% reduction in DON was recorded [212]. In contrast, Bothast et al. [213] observed a low reduction of FB_1 during the fermentation of naturally contaminated maize for ethanol production. Ezekiel et al. [214] reported high percentage (99%, 100%, 98%, 98%, and 76%) reduction of DON, FB, FUS, MON, and ZEN in fermented Nigerian cereal-based beverages (*kunu-zaki* and *pito*), respectively. However, their result showed a much higher reduction in maize-based beverage (*kunu-zaki*), when compared to sorghum-based beverage (*pito*), because the raw maize was more contaminated than the raw sorghum. This proves that the degree of reduction of mycotoxins in foods or feeds is dependent on the initial mycotoxin concentrations. In a recent study on the effect of malting process on *Fusarium* mycotoxins, the authors observed a similar behaviour of DON, 3-ADON, and 15-ADON throughout the malting process, while steeping reduced the concentration of DON, 3-ADON, and 15-ADON between 15%-49% of the initial level, independent of the cultivar and inoculation type [215,216]. In contrast, Kostelanska et al. [217] and Habler et al., [216]

observed an opposite effect on DON-3G after germination, apparently because of the induction of glycosylation of DON by DON-glycosyl-transferase enzyme during germination [215].

In view of the conflicting data reported by different studies on the effect of processing methods on mycotoxins, there is a need for further studies to harmonise and fully understand the behaviour of mycotoxins. Since there is a high possibility of mycotoxins to be modified during food processing, it is imperative to quantify a wide spectrum of mycotoxins using reliable and sensitive analytical methods to ascertain the actual influence of food processing on mycotoxins.

1.6 Analytical techniques for determination of mycotoxins

Detection and quantification of mycotoxins in food and feed commodities have advanced over the last decades. Apparently, because of the wide structural diversity of these chemical compounds and the heterogeneity of complex food and feed matrices, there is a need for more sensitive, robust, and reliable methods. Prior to any analysis, proper sampling procedures are a pre-requisite to obtain reliable results. The accuracy of decisions taken, concerning the actual levels of mycotoxins in bulk of a commodity is determined by measuring the mycotoxin in a representative small portion of the bulk lot [218]. In 2001, the FAO and the WHO recommended that a sub sample of 200 g should be taken from each 200 kg of bulk product [219]. To ensure proper sampling, the EU also laid down methods (401/2006/EC) of sampling and analysis for official control of the levels of mycotoxins in foodstuffs, which involve multiple collection of samples, mixing, and then subdividing until the desired laboratory sample is obtained [220]. Contrary to the state-of-art in EU, there are no established sampling protocols available in SSA. Most of the studies carried out in this region apply the European sampling method [34,166,221]. Subsequently after sampling, samples are required to be pre-treated before determination of mycotoxins using separation and detection methods. It is imperative to highlight that the efficiency and reliability of the result generated by any detection method is dependent on the efficiency of the sample pre-treatment approach employed.

1.6.1 Sample extraction and clean-up method

The choice of pre-treatment used for the extraction of mycotoxins from a matrix depends on the properties of the targeted compound as well as the complexity of the matrix. Since mycotoxins are polar compounds, extraction is

often done by polar solvents or a mixture of polar solvents and water. Currently, the use of chlorinated chemicals for extraction have been discouraged because of their ecological hazards, while the frequently used solvent includes methanol and acetonitrile. In addition to the solvents, other additives such as acids (formic and acetic acid), sodium chloride, and magnesium sulphate are added to the extraction solvent to improve the extraction efficiency, as well as to remove impurities [222]. Application of several extraction approaches such as mechanical shaking, homogenisation, and ultrasonication have also been employed with different purification methods. Available purification methods include liquid–liquid extraction (LLE), column chromatography (CC), supercritical fluid extraction (SFE), solid phase extraction columns (SPE), solid phase micro extraction (SPME), immuno-affinity columns (IAC), as well as one-step multifunctional clean-up columns (Mycosep®). However, those commonly used include LLE, SPE, and IAC [223–225].

According to Turner et al. [225], LLE involves exploiting the solubility of the toxin in an aqueous phase and in an immiscible organic phase, to extract the compound into one solvent leaving the rest of the matrix in the other. However, there is a need for an additional step to remove the non-polar contaminants such as lipids and cholesterol with solvents (hexane or cyclohexane). LLE is effective for determination of several toxins, however, its drawback lies within the duration of time and volume of solvent required as well as the possible loss of sample by adsorption onto the glassware. In recent years, there have been an increasing use of IAC and SPE for clean-up. IAC and SPE require a sequence of three to four steps: preconditioning, sample loading, washing of unwanted compounds, and eluting of the analytes of interest. IAC and SPE have multiple advantages over LLE, such as the elimination or partial elimination of interferences during chromatographic detection, thus providing better detection results. In addition, the use of considerably less solvent, ease of application, short operation time/procedure steps placed them in forefront for use.

Other cheaper novel clean-up methods (e.g. molecularly imprinted polymers (MIPs) and immune-ultrafiltration (IUF)) compared to IAC have been employed for mycotoxin detection [226]. MIPs technique is based on the formation of specific recognition sites in the synthesised polymer. MIPs possess selective and specific cavities for recognition which enables the target analyte to specifically bind to the polymers. After monomer polymerization with a high degree of cross-linking, the target analyte can be eluted by solvent extraction [226]. Beside the low cost, easy and faster application, MIPs have good environmental, thermal, and chemical stability. The IUF method involves the use of an ultra-filtration membrane device with a small pores size enough to retain antibodies. Extraction mixtures pass through the membrane and the target analyte binds to the antibodies. After washing to remove unbound interfering

compounds, the antigen-antibody complexes are dissociated by adding an organic modifier and the analyte is eluted by centrifugation. In view of the different clean-up methods listed above, it is important to mention briefly the evolving high-throughput multiresidue method termed QuEChERS, which have been applied in sample pre-treatment for the determination of a wide range of mycotoxins [227]. QuEChERS as the name implies mean a quick, easy, cheap, effective, rugged, and safe method. Gong et al. [227] reported the application of QuEChERS technique in the determination of 15 mycotoxins with the limit of detection (LOD) ranging from 0.007 µg/kg to 5 µg/kg. This technique which was first applied in the determination of pesticide residuals by Anastassiades et al. [228,229] involves liquid-liquid partitioning using acetonitrile and purifying the extract using dispersive solid-phase extraction. In addition to the determination of wide range of toxins, the QuEChERS has proved to be effective for the determination of mycotoxins in different food and feed matrices [227,230].

1.6.2 Detection methods

Different detection techniques based on the principles of immunoassay, chromatography, and mass spectrometry have been applied for mycotoxin determination and quantification in food and feed commodities. Enzyme-linked immunosorbent assay (ELISA) is the most commonly used immunoassay-based method, probably because of its relatively low cost and easy application. It is based on the specific binding of immunoglobulin antibodies with their specific antigens. This method has been extensively used in SSA. Mbugua and Gathumbi [231] reported the presence of FBs, TH, and ZEN in Kenyan lager beer using ELISA. The incidence of ZEN, DON, T-2, and FBs in cereals and peanuts using ELISA method has been reported by several authors in different parts of SSA [163,232–237]. Although it has been used as a screening tool for different mycotoxins in different matrices, its drawback is often the problem of cross-reactivity and false positives suggesting the need for a confirmatory method. It is also important to mention that the detection of mycotoxins by ELISA is only restricted to a few toxins (specifically the free mycotoxins). Other rapid methods used include biosensors which involve the use of nano-materials (antibodies, enzymes, nucleic acids, cells and tissues) and lateral flow devices [226]. Another analytical method which has been applied for screening purposes is thin layer chromatography (TLC). TLC is one of the oldest and most popular analytical technique used, especially in the developing countries because of its high through-put, low operating cost and ease of identification of the target compound [225]. Although the application of this method has been limited in the developed countries because of the lack in automation, poor separation, low accuracy and sensitivity [238,239], this method still

champions mycotoxin determination in SSA. Application of TLC for *Fusarium* mycotoxin determination in cereal and cereal-based products as well as legumes has been reported [164,240–242].

Other confirmatory methods used for mycotoxin determination include gas chromatography (GC) coupled with mass spectrometry (MS) or flame ionization detection (FID), high performance liquid chromatography (HPLC) coupled with ultra-violet (UV) detection, fluorescence detection (FD), or MS detection. Recently, liquid chromatography-mass spectrometry (LC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) has become very popular for mycotoxin analysis. Apparently, this came as a result of the development of atmospheric pressure chemical ionization (APCI), electrospray ionisation (ESI), and atmospheric pressure photo ionization (APPI) interfaces for LC-MS coupling and the developments in the field of mass analysers. LC-MS/MS compared to other conventional detection techniques, is known for its highly reliable analytical confirmation, its compatibility with a wide range of compound polarities as well as its provision of structural information for further identification [243]. The combination of LC and MS/MS provides an ideal hyphenated technique for the separation and identification of analytes in mixtures which cannot be achieved to this degree by one of these methods alone [244]. MS/MS instruments are mainly used based on either triple quadrupole, ion trap, and Time of Flight analysers (TOF). The possibility to determine multi-mycotoxins in a single chromatographic run using LC-MS/MS counts a major breakthrough. The ionisation sources (APCI or ESI) in LC-MS/MS enable ionisation in both positive and negative mode as well as switching between them in the same chromatographic run, thus giving room for detection of multiple mycotoxins. Studies from different parts of the world on the occurrence of multi-mycotoxins (both free and modified mycotoxins) in food and feed commodities [26,34,166,170,230,245–249], as well as biological materials [245,250,251] using LC-MS/MS have been reported. Of interest is the recent study by Malachova et al. [252], which detected and quantified 295 metabolites (including fungal and bacterial metabolites) in four food matrices in a single chromatographic run. Recently, reports have revealed the application of LC-MS/MS for *Fusarium* mycotoxin determination in studies emanating from SSA [34,166,167,170,214,253–257]. However, it is worth mentioning that most of these studies were carried out in laboratories in the developed countries, thus buttressing lack of infrastructural facilities required to conduct such studies in SSA. Other issues include the insufficient or lack of adequately trained personnel, as well as limited research investments in terms of funding in SSA research centres and academic institutions.

In amidst of several advantages of LC-MS/MS, the analytical challenge remains the inability to screen and identify unknown compounds because of no prior information about these compounds are available. Thus, there is a need

for the development of more sophisticated instrument such as high-resolution mass spectrometry (HR-MS). HR-MS provides full scan spectral information which can give an insight of the overall composition of a sample. It has the advantage of a high mass-resolving power to provide selectivity and the capability for accurate mass measurements which aid identification and provide the possibility for retrospective data analysis [258]. Application of HR-MS for the determination of multi-mycotoxins especially modified mycotoxins as well as identification and elucidation of new metabolites have been reported [27,69,118,119,124,130,132,205,259–262].

1.7 Conclusion

This chapter reviewed the origin, chemistry, distribution, as well as the possible detection techniques employed for the determination of *Fusarium* mycotoxins and their modified forms. The status of mycotoxin research in sub-Saharan Africa (SSA) as well as mitigation strategies during processing with particular reference to *Fusarium* mycotoxins were reviewed. Although there is a wealth of information on *Aspergillus* mycotoxins, especially the aflatoxins in SSA, the reverse remains the case with the *Fusarium* mycotoxins as revealed in this review. The knowledge gap as regards to *Fusarium* mycotoxin research in SSA is of concern because of the frequent occurrence and co-occurrence of these toxins in staple food and food products. Few studies conducted on the occurrence of the major *Fusarium* mycotoxins (FBs, TH, and ZEN) [169] in food and food products in SSA revealed possible high levels of these toxins, in most cases exceeding the maximum limit set by regulatory agencies. Recent concern is the occurrence of emerging and modified *Fusarium* mycotoxins in food and feed commodities. Although the metabolic fate of modified mycotoxins still remains a matter of scientific discourse, SSA should not be left behind. Existing reports on *in vitro* and *in vivo* metabolic studies of modified mycotoxins prove that these toxins may be hydrolysed to the free toxins in the gastrointestinal tract thereby indicating potential toxic relevance on the host species. As such, there is need for a constant and continuous monitoring of the occurrence of *Fusarium* mycotoxins and their modified forms in food and feed commodities as a pre-emptive strategy against outbreak of mycotoxicosis while improving the food and feed quality in SSA.

1.8 References

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STUDY RATIONALE AND OBJECTIVES

CHAPTER 2: STUDY RATIONALE AND OBJECTIVES

2.1 Nigeria: study location

The Federal Republic of Nigeria is located in the West of Africa and lies between Latitude 4° to 14° North and between Longitude 2°2' and 14°30' East. It is bounded by Niger Republic in the North, Chad and Cameroon in the East, Benin Republic in the West, and Gulf of Guinea in the South. Nigeria, the number one most populated country in Africa has a total land mass of 923,768 km^2, and is endowed by its cultural diversity with over 250 ethnic groups and languages. Culture transmits the way of life of a society, influences the roles, uses, position, symbolism of individuals, ideas, and objects. The culture of a place is intrinsically linked to the food consumed in the region as culture prescribes the interaction between people, between people and land, and between land and food. Ethnicity is also related to food security because the environments where people live and their ancestral origins influence the food culture and practices passed on from one generation to another. The cultural diversity of Nigeria gave birth to a diversity of food systems which are also influenced by several environmental factors such as soil, rainfall, and temperature.

Nigeria's climate is characterised by strong latitudinal zones, becoming progressively drier as one moves towards the North from the coast. Rainfall is the key climatic variable, and there is a marked alternation of wet and dry seasons in most areas [1]. Nigeria is divided into 36 states and 7 agro-ecological zones (AEZs) in respect to the farming systems (Figure 2.1) [2]. The AEZs include Derived Savanna (DS), Humid Forest (HF), Southern Guinea Savanna (SGS), Northern Guinea Savanna (NGS), Sahel Savanna (SaS), Mid Altitude (MA), and Sudan Savanna (SS). The geographical location, temperature, and rainfall patterns of the DS, SGS, NGS, SS, and HF are documented by Udoh et al. and Atehnkeng et al. [3,4]. These zones are the major agricultural producing areas and as such were the sampling zones considered in this PhD study. Briefly, the DS is characterised by an annual rainfall distribution and temperature ranging between 1300 mm and 1500 mm, and 25 °C to 35 °C, respectively. The average annual rainfall of SGS is between 1000 mm and 1300 mm with a temperature range of 26 to 38 °C. The NGS has a rainfall distribution averaging between 900 mm and 1000 mm annually, and temperatures variation of 28 to 40 °C, while the SS is characterised by an annual rainfall distribution of between 650 mm and 1000 mm annually, and temperatures variation of 30 to 40 °C. The HF has an average annual rainfall of between 1,300 mm and 2,000 mm and a temperature range of 26-28 °C.

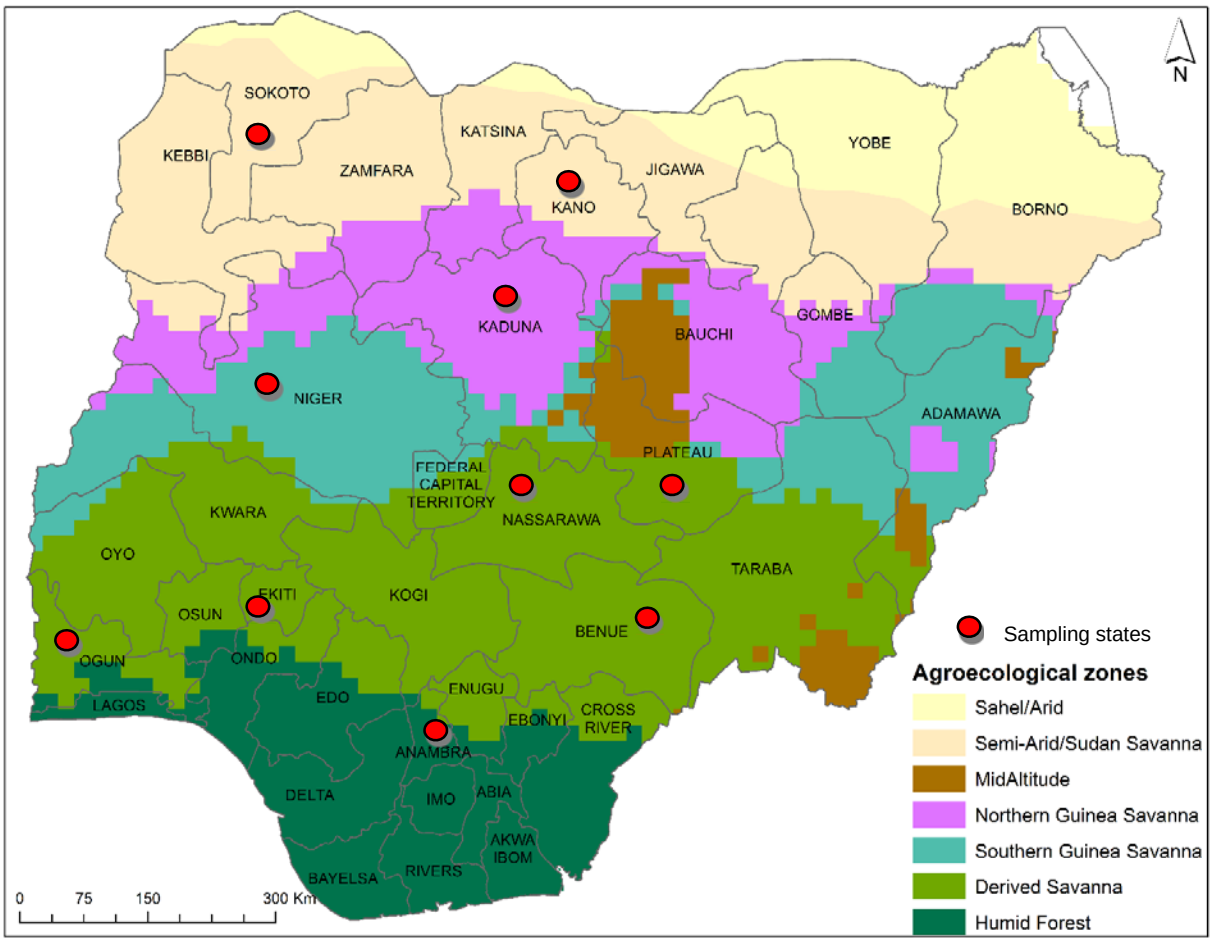

Figure 2.1. Map of Nigeria showing the different agroecological zones and all the sampling states considered in this Phd study.

Nigeria is referred to as giant of the African continent because of its fastest growing economy with a gross domestic product (GDP) worth of 405.10 billion US dollars in 2016, which represented 0.65 percent of the world economy. Despite the potentials and vast natural resources of Nigeria, it ranks one of the top countries affected by food insecurity and food safety. This may be attributed to several factors such as terrorism, unemployment, natural disasters, corruption, political instability, and a weak agricultural sector.

2.2 Study Rationale

As food security and safety issues pilot discussion worldwide, SSA especially Nigeria must not be left behind. These concepts which are interrelated with profound impact on the quality of human life remain a major problem in Nigeria. According to FAO [5] food security is determined by the availability and accessibility to sufficient nutritious foods, as well as the utilisation of the available foods in respect to cultural perspectives. However, because of the problem of food insufficiency and rapid increase of the population in SSA, food quality and safety are often compromised. This is worsened as food safety issues are not prioritised. As a result, a large population of the over 180 million Nigerians are being exposed to a wide range of chemical hazards with varying detrimental health effects.

Mycotoxins remain a major food safety concern ravaging SSA. These toxic secondary metabolites contaminate virtually all agricultural crops, and are produced by several genera of fungi including *Aspergillus, Fusarium, Penicillium,* and *Alternaria.* Of the mentioned genera, SSA including Nigeria has paid more attention to *Aspergillus* species (spp.) and their mycotoxins, especially aflatoxins, due to their link with the tropical climate, thus the existence of mainly regulatory limits for aflatoxins in some countries of the region. Unfortunately, the present climatic change scenarios have been predicted to have significant effects on the ecosystem of these organisms, thus other fungal genera and their secondary metabolites should not be neglected. Of a great importance are the *Fusarium* fungi, which have the potential to infest agricultural plants in the field causing several devastating diseases to crops. These fungi also have the ability to produce a wide diversity of mycotoxins either in the field or during storage under favourable environmental conditions. Exposure to this group of metabolites have been reported in different parts of the world. Although, evidence of frequent occurrence of major mycotoxins producing *Fusarium* fungi such as *F. verticillioides*, *F. graminearum*, *F. poae*, *F. proliferatum*, and *F. sporotrichioides* in Nigerian food commodities have been reported [6–8], this group of toxins has been ignored leading to depletion of information on the occurrence of *Fusarium* mycotoxins and their modified forms in agricultural commodities and products. Also, no regulations protecting consumers against these toxins exist in Nigeria.

Notwithstanding the present situation of research in Nigeria, food commodities from this region are at high risk of contamination by these toxins [9]. The impact of *Fusarium* mycotoxins in Nigeria is yet to be ascertained. It is believed that these toxins burden a relatively weak economy as well as the health of the populace due to loss of food crops and exposure of humans and animals to acute and chronic diseases. The situation is made complex with the emergence of structurally modified secondary metabolites. Pragmatic intervention strategies will seek to bring stakeholders including farmers and food producers to target the reduction of these toxins at every point in the

production or processing chain of food products. Increased research and development such as use of seeds resistant to fungal attack, improved technologies, and manoeuvring of processing conditions to give significant reduction of mycotoxins in final products have been encouraged. Monitoring the occurrence of *Fusarium* mycotoxins and their modified forms in Nigerian staple crops and processed food products therefore becomes imperative. While there are studies on the fate of *Fusarium* toxins during processing of diverse food products in other parts of the world, the occurrence and behaviour of *Fusarium* mycotoxins during processing of Nigerian foods and products is very scarce or non-existent. It is therefore timely and important to examine the status of *Fusarium* mycotoxins in Nigerian staple food as well as to understand the influence of different indigenous processing methods on the behaviour of *Fusarium* mycotoxins and their modified forms during production of traditional foods in Nigeria.

This study aims to investigate the incidence of *Fusarium* mycotoxins and their modified forms in staple crops and indigenous food products consumed by the Nigerian population. Additionally, the study aims to investigate the fate of *Fusarium* mycotoxins during the traditional processing of the indigenous food products using fermentation, boiling, and roasting processes. The goals of this study were achieved within the following objectives:

Objective 1 – to critically review the status of *Fusarium* mycotoxin research in SSA as it regards to occurrence, emerging trends, and post-harvest mitigation control strategies towards food control (Chapter 1).

Objective 2 – to investigate the occurrence of *Fusarium* mycotoxins and their modified forms in staple crops (maize, sorghum, millet, soybean, African locust bean, mesquite seed, and castor bean seed) and locally processed food products (*ogi,* soybean powder, *pito, burukutu, dawadawa*, *ogiri, okpehe, garri*, *lafun,* and *amala*) from Nigerian markets (Chapters 3, 4, and 5).

Objective 3 – to assess the influence of traditional processing (fermentation, roasting and boiling) methods of infant meal (*ogi* and soybean powder), traditional beverages (*pito* and *burukutu*), and traditional spices (*dawadawa, ogiri*, and *okpehe*) on the occurrence of *Fusarium* mycotoxins and their modified forms (Chapters 6 and 7).

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OCCURRENCE OF *FUSARIUM* MYCOTOXINS IN CEREALS AND CEREAL-BASED PRODUCTS FROM NIGERIA

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Chapter 3: OCCURRENCE OF *FUSARIUM* MYCOTOXINS IN CEREALS AND CEREAL-BASED PRODUCTS FROM NIGERIA

3.1 Introduction

Cereals such as maize (*Zea mays*), sorghum (*Sorghum bicolor*), and millet (*Pennisetum glaucum*) serve as major staple crops consumed especially by the middle and low-income earners in Nigeria. These crops are often processed into different food forms including traditional weaning meals and traditional fermented beverages in the region. *Ogi* (also known as *akamu, koko,* or pap) is a fermented cereal-based product used as a major traditional weaning food for infants, food for the convalescent and the elderly, as well as consumed by different age groups especially as breakfast meal in Nigeria. It is produced by submerge fermentation of cereal grains (maize, sorghum, or millet) for two to three days followed by wet milling and sieving through a mesh. The fermentation process of *ogi* is usually initiated by chance inoculation under uncontrolled environmental conditions thereby resulting in variable quality of the final product (see Chapter 7 for more details on *ogi* processing). Regardless of the microbial diversity during the production of *ogi*, studies have reported the dominance of yeast especially *Saccharomyces cerevisiae* and lactic acid bacteria (LAB) especially *lactobacillus* (*L. plantarum* and *L. fermentum*), *leuconostoc mesenteroides,* and *Pediococcus* spp [1–4]. Besides these microorganisms, several other moulds belonging to the genera of *Aspergillus*, *Fusarium,* and *Penicillium* have also been isolated during *ogi* fermentation [1,3,4].

Another important cereal-based product is traditional beer. Traditional beer is significantly consumed by millions of Nigeria people. It is noteworthy to mention that the consumption trend is higher in poor resource communities due to ease of affordability and relatively high cost of bottled/canned commercial beers. *Pito* and *burukutu*, which rank the major traditional fermented beers in Nigeria, are mostly prepared by women using cereals (mostly sorghum and millet) (see Chapter 6 for more details on *pito* and *burukutu* processing), and oftentimes serves as a channel of disposing poor-quality grains by the brewers (A. Jibril, personal communication, January 18, 2017). Similar to *ogi*, *pito* and *burukutu* are produced through spontaneous fermentation resulting to a wide diversity of microorganisms. The quality of the raw material also plays an important role in determining the fermenting microorganisms. Atter et al. [5] reported LAB (*L. plantarum* and *L. fermentum*) and yeast (*S. cerevisiae*) as the major fermenting microorganisms contributing to the characteristic sour taste and alcohol content of the final product, respectively. Other microorganisms such as *Escherichia* spp., *Staphylococcus* spp., *Bacillus* spp., *Streptococcus* ssp., *Aspergillus* ssp., and *Rhizopus* have also been isolated [6,7].

Studies have reported the prevalence of *Fusarium* mycotoxins, particularly TH, ZEN, and FBs, in cereal crops and cereal-based products globally [8–11]. In most cases, these mycotoxins may co-exist in food and food products which often results to a synergistic, additive, or antagonistic toxic effect on the host [12]. Recently, issues of possible co-existence of these *Fusarium* mycotoxins and their modified forms have become of great concern. The possible co-occurrence of *Fusarium* mycotoxins and modified mycotoxins such as DON-3G, ZEN-14G, α-zearalenol-4-glucoside (α- ZEL-4G) and β-zearalenol-4-glucoside (β-ZEL-4G) have been reported in a wide of range of cereals and cereal-based products [9,13]. In spite of this obvious evidence, limited study has been undertaken to ascertain the occurrence of *Fusarium* mycotoxins and their modified forms in Nigerian food products. The paucity of information has resulted to the lack of regulatory maximum levels governing the control of *Fusarium* mycotoxins in Nigeria.

This novel study investigates the occurrence of a wide profile of *Fusarium* mycotoxins and their modified forms including FB_1, FB_2, and FB_3, modified FB, DON, 3-ADON, 15-ADON, DON-3G, ZEN, α-ZEL, β-ZEL, ZEN-14G, NIV, FUS-X, T-2, HT-2, DAS, and NEO in Nigerian cereals: maize, sorghum, millet, and their processed products (*ogi, pito,* and *burukutu*).

3.2 Materials and methods

3.2.1 Sampling

The survey sites for this study were considered based on the production areas of the crops and products. One state from each agro-ecological zone was covered for each crop and product. Maize samples were collected from four AEZs (SGS, NGS, SS, DS); millet and sorghum were sampled from SGS, NGS, and SS; *burukutu* and *pito* were sampled from DS and NGS; while *ogi* samples were collected from DS. Figure 3.1 and Table 3.1 show the detailed sampling sites, while Annex 2.1 depicts some pictorial sampling memories. The geographical location, temperature, and rainfall pattern of DS, SGS, NGS, and SS are documented by Udoh et al. and Atehnkeng et al. [14,15] and are briefly described in Chapter 2, Section 2.1.

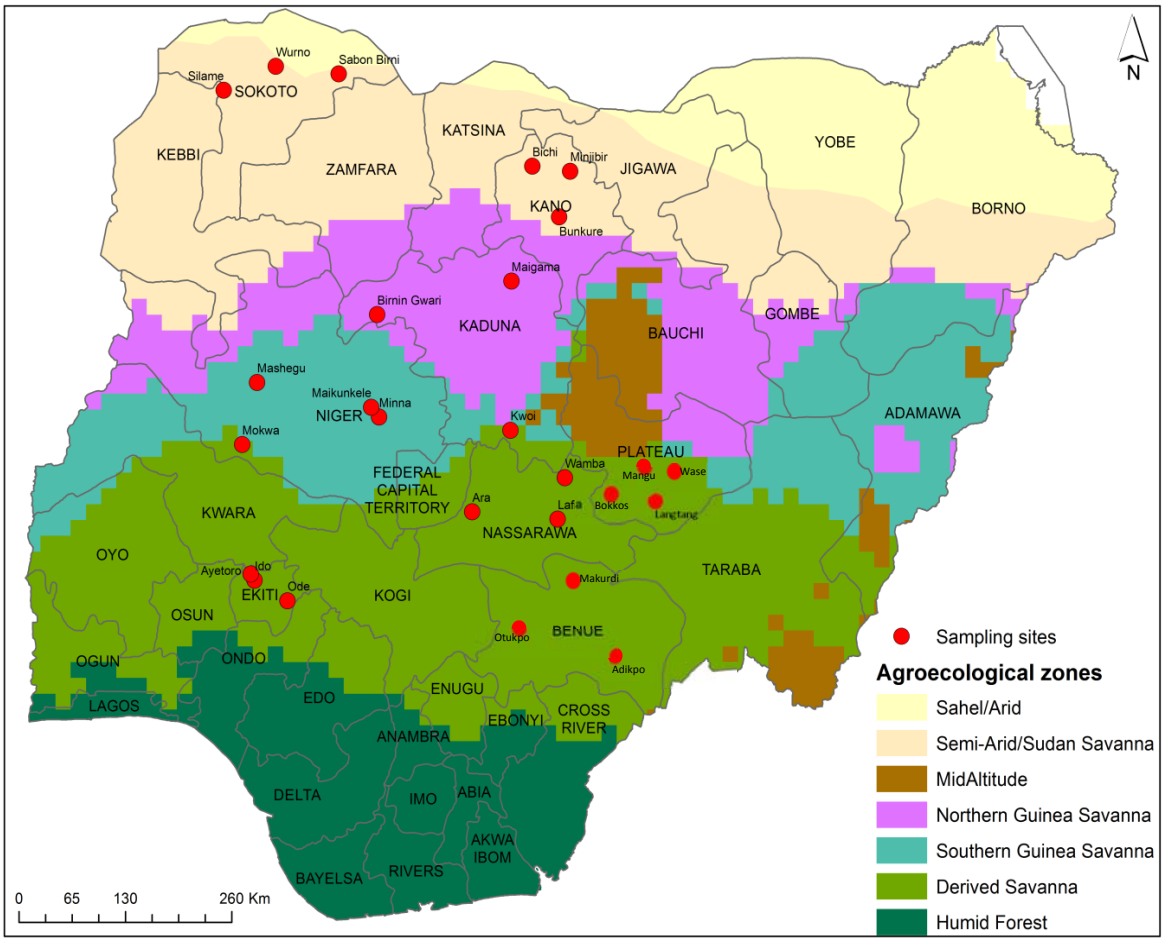

Figure 3.1. Sampling locations of maize, sorghum, millet, *burukutu, pito*, and *ogi* according to the different agro-ecological zones

A total of 462 samples comprising of maize (*n* = 136), sorghum (*n* = 110), millet (*n* = 87), *burukutu* (54), *pito* (45), and *ogi* (*n* = 30) were randomly collected from selected markets between September 2015 and October 2015. The cereal samples were clean grains with no visible mould. Sampling was carried out as described by Adetunji et al. [16] and the European Commission Regulation with some modifications [17].

Table 3.1. Sample distribution of food commodities (maize, sorghum, millet, *burukutu, pito*, and *ogi*) from various agro-ecological zones of Nigeria.

Product Type	AEZs	State	No. of Markets	No. of Samples
Maize	SS	Kano	6	30
	NGS	Kaduna	6	36
	SGS	Niger	8	40
	DS	Nasarawa	6	30
				Total number = 136
Sorghum	SS	Kano	6	30
	NGS	Kaduna	2	40
	SGS	Niger	8	40
				Total number = 110
Millet	SS	Sokoto	6	30
	NGS	Kaduna	6	30
	SGS	Niger	8	27
				Total number = 87
Burukutu	NGS	Kaduna	6	30
	DS	Benue	6	24
				Total number = 54
Pito	NGS	Kaduna	6	15
	DS	Plateau	8	30
				Total number = 45
Ogi	DS	Ekiti	5	30
				Total number = 30

AEZs = agro-ecological zones, SS = Sudan Savanna, NGS = Northern Guinea Savanna, SGS = Southern Guinea Savanna, DS = Derived Savanna.

Briefly, the whole content of a traditional bag of 50 kg of maize, sorghum, and millet was considered as a lot. An aggregate sample size of 1 kg was composed of 5 incremental samples. Each incremental portion was about 200 g, and one was taken from different positions in the bag. In the case of *ogi*, 300 g of sample was collected from the top, middle, and bottom portions of the jute sack of 10 kg *ogi*, thoroughly mixed together, air dried, and packed in a plastic container. While for the traditional beers, 500 mL of each of the sample was collected from a thorough homogenised pot (20 L) of traditional beer (either *burukutu* or *pito*), packed in a plastic bottle, and temporary stored at -20 °C. Prior to shipment of samples to the Laboratory of Food Analysis (Ghent University, Belgium), each sample of the different food products (maize, sorghum, millet, *burukutu, pito*, and *ogi*) was thoroughly homogenised. A representative portion of 200 g for the dried food products and 200 mL for the traditional beers was taken, labelled, and sealed in zip lock bags or plastic bottles, respectively, for further analysis. Prior to analysis, maize, sorghum,

millet, and *ogi* samples were milled to a sieve size of 0.5 to 1 mm using an IKA M20 universal mill (Sigma-Aldrich, Bornem, Belgium).

3.2.2 Chemicals and reagents

Methanol (MeOH, LC-MS grade), glacial acetic acid (LC-MS grade), and analytical grade acetonitrile were purchased from Biosolve B.V. (Valkenswaard, The Netherlands). Analytical grade acetic acid, ammonium acetate and sodium chloride were obtained from Merck (Darmstadt, Germany). Analytical grade of n-hexane and methanol, and Whatman® glass microfiber filters (GFA, 47 mm diameter) were purchased from VWR International (Zaventem, Belgium). Ultrafree®-MC centrifugal filter devices (0.22 µm) were obtained from Millipore (Bredford, MA, USA). C18 SPE columns and MultiSep®226 AflaZon+ multifunctional columns were purchased from Alltech (Lokeren, Belgium) and Romer Labs (Gernsheim, Germany), respectively. Anhydrous magnesium sulphate (MgSO4) was purchased from Sigma-Aldrich, Steinheim, Germany. Water was purified using a Milli-Q Gradient System (Millipore, Brussels, Belgium). All other chemicals and reagents used were of analytical grade.

The analytical mycotoxin standards including FB_1, FB_2, DON, 3-ADON, 15-ADON, deepoxy-deoxynivalenol (DOM), FUS-X, NIV, HT-2, NEO, ZEN, zearalanone (ZAN), α-ZEL, β-ZEL were purchased from Sigma-Aldrich (Bornem, Belgium). FB_3 was obtained from Promec Unit (Tygerberg, South Africa). DAS, DON-3G, and T-2 were purchased from Biopure Referenzsubstanze (Tulln, Austria). Hydrolysed fumonisin B_1 ($HYFB_1$) was from Romer Labs®, Tulln, Austria, while ZEN-14G was synthesized via an in-house validated method according to Zill et al. [18]. Stock solutions of FB_1, FB_2, FB_3, DON, 3-ADON, 15-ADON, HT-2, T-2, ZEN, ZAN, α-ZEL, β-ZEL, FUS-X, NIV, NEO, and DAS were prepared in MeOH at a concentration of 1 mg/mL. DOM (50 µg/mL), DON-3G (50.2 µg/mL), and ZEN-14G (100 µg/mL) were obtained as solution in acetonitrile. All stock solutions were stored for 1 year or until the expiration date at −18 °C. The working standard solutions were made by diluting the stock standard solutions in methanol, and were stored at −18 °C for 3 months. From the individual stock and working standard solutions, a standard mixture was prepared in methanol at the following concentrations: FB_1, FB_2, and DON (40 ng/µL), FB_3 (25 ng/µL), 3-ADON (5 ng/µL), 15-ADON (2.5 ng/µL), HT-2, T-2, ZEN, α-ZEL, β-ZEL, ZEN-14G, DON-3G, and NEO (10 ng/µL), FUS-X and NIV (20 ng/µL), and DAS (0.5 ng/µL). The mixture was stored at −18 °C, and renewed every 3 months.

3.2.3 Sample extraction

For the dried samples (maize, sorghum, millet, and *ogi*), sample preparation for 17 *Fusarium* mycotoxins was carried out as described by Monbaliu et al. [19] for multi-mycotoxin analysis. Briefly, 5 g of sample was spiked with internal standards (ZAN and DOM at a concentration of 250 and 150 µg/kg, respectively). DOM was used as internal standard for DON, DON-3G, 3-ADON, and 15-ADON while ZAN was used for the other mycotoxins. The spiked sample was kept in the dark for 15 min and extracted with acetonitrile/water/acetic acid (79/20/1, v/v/v). The supernatant was passed through a preconditioned C18-SPE column, and the extract was defatted. In order to recover the 17 *Fusarium* mycotoxins, two clean-up pathways were followed. First, 12.5 mL of the defatted extract was added to 27.5 mL of acetonitrile/acetic acid (99/1, v/v), and passed through a MultiSep®226 AflaZon+ multifunctional column. In the second pathway, 10 mL of defatted extract was filtered using a glass microfilter. Two mL of the filtered extract were combined with the Multisep226 eluate, and evaporated to dryness.

For the traditional beer samples, a QuEChERS method was used for the extraction of the *Fusarium* mycotoxins listed above, including $HYFB_1$. Briefly, 5 mL of beer samples were degassed by sonication for 10 min, and spiked with internal standards ZAN and DOM at a concentration of 250 µg/kg and 150 µg/kg, respectively. Spiked samples were kept in the dark for 15 min; and 2 g of $MgSO_4$ and 20 mL of acetonitrile/acetic acid (99/1, v/v) were added. Samples were vortexed and agitated vigorously for 1 h at 93 rpm using an overhead shaker. The mixture was then centrifuged for 10 min at 4000 g, and the supernatant was filtered through a glass microfiber filter into an extraction tube followed by drying under a gentle nitrogen flow in a thermostatic water bath heated at 40 °C.

The dried residue was reconstituted in 150 µL injection solvent containing of water/methanol/acetic acid (94/5/1, v/v/v) + 5 mM ammonium acetate and water/methanol/acetic acid (2/97/1, v/v/v) + 5mM ammonium acetate mixed in the ratio of 3/2, v/v. Further filtration was done for 15 min at 10,000 g using the Ultrafree ® centrifuge filter (0.5 mL, pore 0.22 µm, Millipore Bedford, MA, USA) prior to injection into the LC-MS/MS system.

3.2.4 Preparation of the hydrolysed FB_1, FB_2, and FB_3 standard

Before the purchase of $HYFB_1$ from Romer Labs® (Tulln, Austria) as mentioned in Section 3.2.2, the hydrolysed fumonisins (HYFBs) standards used in this PhD study were prepared as described by Dall Asta et al. [20]. Briefly, 90 µL standard solution of FB_1, FB_2, and FB_3 (50 µg/mL of each) was prepared in methanol, transferred to a 10 mL sovirel tube, and evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 2 M NaOH (1 mL),

and the reaction was incubated overnight at room temperature. After hydrolysis, the mixture was extracted by liquid-liquid partitioning using acetonitrile (1 mL). The extraction was repeated two more times using 1 mL fresh acetonitrile. The organic phases were pooled together, evaporated under a nitrogen stream and redissolved in 1 mL of methanol. The reaction yield was checked by LC-MS, by monitoring the conversion of FBs to HYFBs and the absence of side-products. Calibration curves were prepared by proper dilution of the standard solution, assuming the total conversion of the native compounds to the hydrolysed forms. Figure 3.2 shows the chromatograms obtained with the synthesised HYFBs standards.

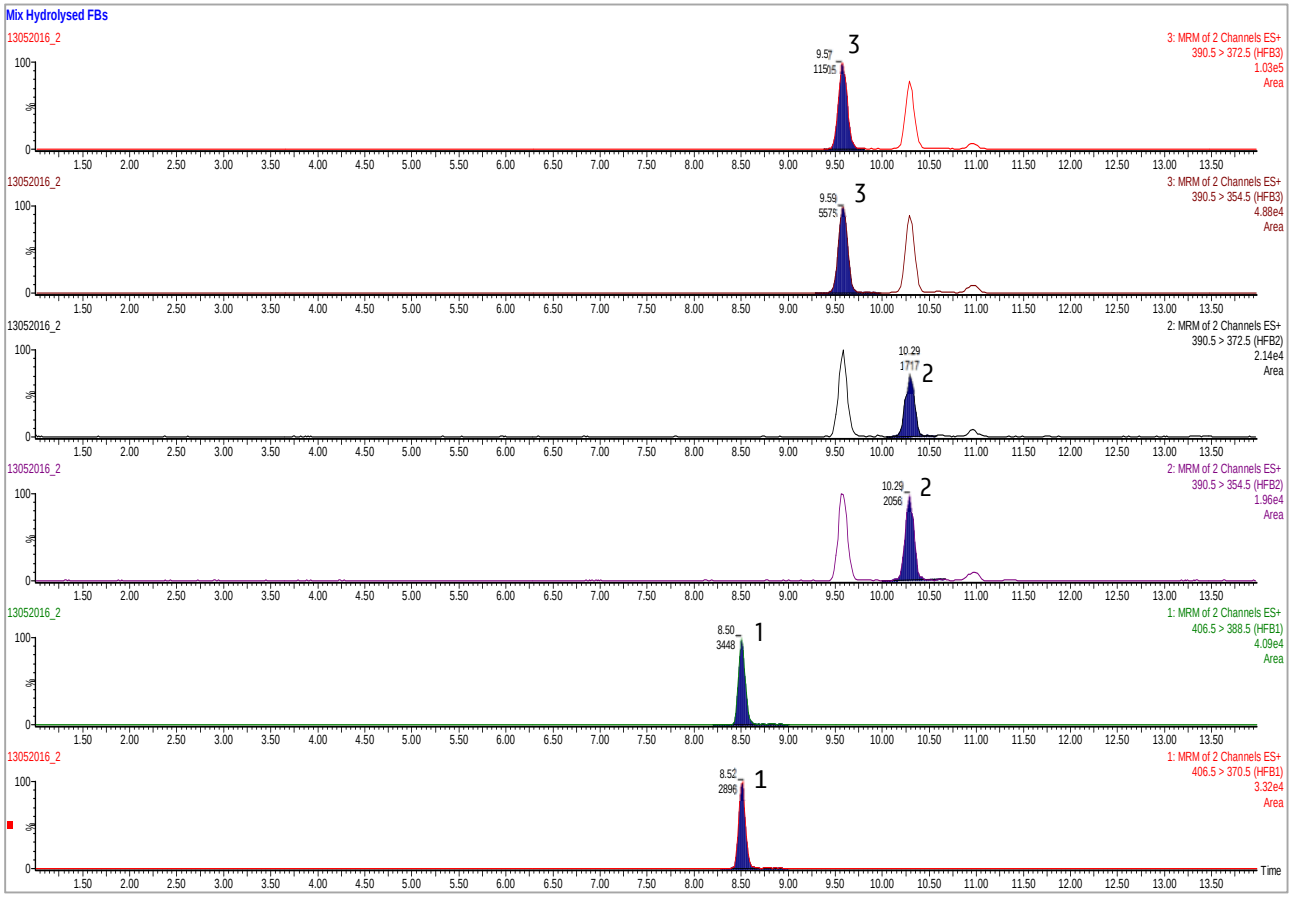

Figure 3.2. Chromatograms showing quantifying and qualifying ions obtained with a mixture of hydrolysed fumonisin B_1 (1), B_2 (2), and B_3 (3) standard.

3.2.5 Sample preparation for hydrolysed fumonisins

Ten samples from each dried matrix (maize, sorghum, millet, and *ogi*) were selected for analysis of HYFBs. Five samples comprised of high FBs-contaminated samples, and another five samples were negative samples. Sample preparation was carried out as described by Dall Asta et al. [20]. Two and a half grams of each sample were weighed, and hydrolysed at room temperature using 50 mL of 2 M NaOH, homogenised with the Ultraturrax for 3 min, and then stirred using a magnetic stirrer for 60 min. Subsequently, 50 mL of acetonitrile was added, and homogenised for 3 min, and 20 mL of the upper organic phase was transferred into a centrifuge tube, and centrifuged for 15 min at 3500 rpm. Then, 4 mL was evaporated, and the residue was redissolved in 1 mL of water/methanol (30/70, v/v), and analysed by LC-MS/MS.

3.2.6 Liquid chromatography-tandem mass spectrometry

A Waters Acquity UPLC system coupled to a Waters Quattro Micro triple-quadrupole mass spectrometer (Waters, Milford, MA, USA) was used for the detection and quantification of mycotoxins in the samples. The data acquisition and processing utilities included the use of the MassLynx™ version 4.1 and QuanLynx® version 4.1 software (Waters, Manchester, UK). The analytical conditions were the same as described by Monbaliu et al. [21]. The column used was a Symmetry C18 (150 mm × 2.1 mm i.d. 5 µm) column with a guard column (10 mm × 2.1 mm i.d.) of the same material (Waters, Zellik, Belgium), and was kept at room temperature. The injection volume was 20 µL. Mobile phase consisting of water/methanol/acetic acid (94/5/1, v/v/v) and 5 mM ammonium acetate (mobile phase A), and methanol/water/acetic acid (97/2/1, v/v/v) and 5 mM ammonium acetate (mobile phase B) was used at a flow rate of 0.3 mL/min with a gradient elution program. The gradient started at 95% mobile phase A with a linear decrease to 35% in 7 min. Mobile phase A decreased to 25% at the next 4 min. An isocratic period of 100% mobile phase B started at 11 min for 2 min. Initial column conditions were reached at 23 min using a linear decrease of mobile phase B, and the column was reconditioned for 5 min using mobile phase A prior to the following injection.

The mass spectrometer was operated using selected reaction monitoring (SRM) channels in positive electrospray ionization (ESI+) mode. More information on the transition of the different mycotoxins are described in Annex 1. The capillary voltage was 3.2 kV, and nitrogen was used as the cone and desolvation gas at 20 and 800 L/h, respectively. Source and desolvation temperatures were set at 150 and 350 °C, respectively. The identification of *Fusarium* mycotoxin positive samples was done based on the fulfilment of the following criteria: a peak with signal to noise ratio of not less than 3, the relative retention time (with regard to the internal standard) of the compound must

range within a margin of 2.5% (maximum permitted deviation), and the deviations of the relative ion intensities of the MRM transitions were not greater than the maximum permitted tolerances [22],

3.2.7 Method validation

For the cereal matrices, validation parameters including apparent recovery, limit of detection (LOD), limit of quantification (LOQ), repeatability, and reproducibility were evaluated. While for the processed products, LOD, LOQ, and apparent recovery were evaluated. Five blank samples of each matrix were spiked in triplicate with the different mycotoxins (FB_1, FB_2 and FB_3, ZEN, α-ZEL, β-ZEL, ZEN-14G, DON, 3-ADON, 15-ADON, DON-3G, NIV, FUS-X, T-2, HT-2, DAS, NEO) on three different days at concentration levels shown in Table 3.2. ZAN was added as internal standards (IS) for FB_1, FB_2, FB_3, ZEN, α-ZEL, β-ZEL, ZEN-14G, NIV, FUS-X, T-2, HT-2, DAS, NEO, while DOM was used as IS for DON, 3-ADON, 15-ADON, DON-3G. Matrix-matched calibration (MMC) plots were constructed by applying the least-squares method, and by plotting the relative peak area (peak area of toxin/peak area of IS) against the spiked concentration level of the sample. The linearity was graphically evaluated using a scatter plot, and the linear regression model was tested using a lack-of-fit test. The apparent recovery for each of the mycotoxins was evaluated by dividing the observed value from the MMC curves by the spiked level. The obtained results ranged between 75% and 110%, which is in conformity with the range set in legislation [23].

A precision study for the cereal matrices with regards to repeatability (intraday precision, RSD_r) and reproducibility (interday precision, RSD_R) within laboratory was performed using five concentration levels, and was calculated using the relative standard deviation (RSD). The results are presented in Table 3.2. LOD and LOQ for individual mycotoxins were obtained from the signal-to-noise (S/N) ratio which have been defined and set as 3 and 6, respectively, by the International Union of Pure and Applied Chemistry (IUPAC). The LODs and LOQs of individual mycotoxins in cereals (maize, sorghum, and millet) and cereal-based products (*ogi, burukutu*, and *pito*) are shown in Table 3.3.

3.2.8. Statistical Analysis

Data were processed and calculated using Microsoft office Excel 2007 (Redmond, WA, USA) and the statistical package R version 3.0.3 (The R Foundation for Statistical Computing, Vienna, Austria). The Kruskal Wallis statistical test (an alternative to one-way analysis of variance (ANOVA) under nonparametric test) was used to assess the significance of the differences between the mycotoxin concentrations and the different AEZ.

Table 3.2 Intraday repeatability (RSD_r) and interday reproducibility (within-laboratory) (RSD_R) of the individual mycotoxins for maize, sorghum, and millet.

Mycotoxin	RSD_r (%)								RSD_R (%)							
	Conc. (µg/kg)	Maize	Sorghum	Millet	Conc. (µg/kg)	Maize	Sorghum	Millet	Conc. (µg/kg)	Maize	Sorghum	Millet	Conc. (µg/kg)	Maize	Sorghum	Millet
FB_1	200	7.2	14.3	13.9	800	3.3	5.2	4.9	200	9.8	11.2	11.5	800	7.5	7.0	8.2
FB_2	200	8.8	12.1	12.1	800	5.5	8.0	6.4	200	15.3	10.5	12.5	800	8.2	9.0	9.4
FB_3	25	17.4	26.1	14.7	100	16.3	22.7	11.5	25	23.1	22.5	25.6	100	25.7	25.5	38.8
DON	200	24.2	18.9	8..1	800	10.7	14.0	7.0	200	20.5	23.7	18.4	800	18.0	20.1	15.2
3-ADON	25	15.5	23.0	14.0	100	13.2	22.4	8.6	25	22.1	17.2	14.2	100	17.0	15.4	10.9
15-ADON	12.5	12.8	16.5	8.4	50	11.5	15.6	4.1	12.5	17.3	19.1	8.5	50	16.4	18.7	6.0
DON-3G	5	18.1	7.5	7.8	20	11.1	6.0	6.0	5	13.2	12.3	15.2	20	9.6	7.4	9.2
ZEN	50	12.3	8.6	12.0	200	6.9	6.0	6.0	50	13.5	14.4	19.4	200	11.2	9.3	11.4
α- ZEL	50	23.1	19.2	18.9	200	17.6	18.2	17.5	50	22.9	22.0	22	200	21.5	22.1	22.4
β-ZEL	50	11.2	11.0	12.1	200	10.0	9.4	10.3	50	11.4	17.2	16.2	200	10.5	13.0	11.4
ZEN-14G	50	21.3	19.5	23.5	200	17.5	23.7	16.1	50	22.4	22.2	15.8	200	21.5	21.5	15.0
NIV	100	21.0	23.1	16.4	400	22.1	23.9	14.0	100	27.0	19.4	17.3	400	24.3	16.1	16.5
FUS-X	100	13.5	11.5	10.6	400	10.0	8.0	9.2	100	24.2	17.1	15.3	400	22.2	14.4	14.0
T-2	50	12.3	9.0	15.2	200	9.4	8.3	13.4	50	26.0	14.2	10.2	200	18.2	10.5	10.3
HT-2	50	8.2	9.2	10.8	200	6.7	8.5	9.7	50	9.2	12.0	10.2	200	7.4	9.5	8.1
DAS	2.5	15.3	14.0	13.2	10	10.1	15.2	12.2	2.5	19.2	17.7	15.4	10	17.6	16.5	13.7
NEO	50	13.6	12.0	12.4	200	11.5	12.4	11.6	50	24.0	24.5	26.5	200	23.5	23.6	23.5

RSD_r = Intraday repeatability, RSD_R = interday reproducibility (within-laboratory), FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3, DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, NIV = nivalenol, FUS-X = fusarenon-X, T-2 = T-2 toxin, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, NEO = Neosolaniol

Table 3.3 Limits of detection (LOD) and limits of quantification (LOQ) of the individual mycotoxins for maize, sorghum, millet, *ogi*, *burukutu*, and *pito*.

Mycotoxin	LOD (µg/kg)						LOQ (µg/kg)					
	Maize	Sorghum	Millet	*Burukutu*	*Pito*	*Ogi*	Maize	Sorghum	Millet	*Burukutu*	*Pito*	*Ogi*
FB_1	8.2	10.0	8.2	10.4	8.0	8.1	16.4	20	16.4	20.8	16.0	16.2
FB_2	11.5	11.3	12.1	12.2	11.1	11.9	23.0	22.6	24.2	24.4	22.2	23.8
FB_3	14.0	14.0	14.0	14.0	14.0	13.7	28.0	28	28	28.0	28.0	27.4
$HYFB_1$	2.4	6.0	3.5	2.4	2.4	2.7	4.8	12	7.0	4.8	4.8	5.4
DON	7.0	12.0	10.0	10.3	7.2	6.0	14.0	24	20	20.6	14.4	12.0
3-ADON	10.4	12.0	10.4	11.4	9.6	3.9	20.8	24	20.8	22.8	19.2	7.8
15-ADON	5.0	7.0	4.4	4.6	5.8	4.3	10.0	14	8.8	9.2	11.6	8.6
DON-3G	15.3	3.8	3.8	3.8	3.8	4.5	30.6	7.54	7.54	7.6	7.6	9.0
ZEN	3.3	3.8	3.8	3.6	3.6	3.2	6.5	7.7	7.6	7.2	7.2	6.4
α-ZEL	4.6	4.6	5.1	4.5	4.4	4.4	9.2	9.2	10.2	9.0	8.8	8.8
β-ZEl	5.0	5.0	7.0	5.0	5.0	5.0	10.0	10.0	14	10.0	10.0	10.0
ZEN-14G	8.0	7.2	8.0	6.8	6.8	7.0	16.0	14.4	16	13.6	13.6	14.0
NIV	35.0	87.5	81.3	44.1	49.3	38.0	70.0	175	162.6	88.2	98.6	76.0
FUS-X	20.6	45.0	73.6	47.8	51.2	22.2	41.2	90	147.2	95.6	102.4	44.4
T-2	3.6	8.0	5.4	3.3	3.6	9.2	7.2	16	10.8	6.6	7.2	18.4
HT-2	6.5	6.5	6.5	3.0	4.0	4.8	13.0	13	13	6.0	8.0	9.6
DAS	0.32	0.5	0.32	0.6	0.9	0.4	0.64	1.0	0.64	1.2	1.8	0.8
NEO	2.2	3.0	3.7	3.2	2.4	2.3	4.4	6.0	7.4	6.4	4.8	4.6

LOD = Limit of detection; LOQ = Limit of quantification. FB_1, B_2, and B_3 = Fumonisin B_1, B_2, and B_3, $HYFB_1$ = hydrolysed fumonisin B_1, DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, NIV = nivalenol, FUS-X = fusarenon-X, T-2 = T-2 toxin, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, NEO = Neosolaniol.

3.3 Results and discussion

3.3.1 *Fusarium* mycotoxin contamination in cereals (maize, sorghum, and millet) and processed products (*ogi, burukutu, and pito*) from Nigeria

Out of the 18 *Fusarium* mycotoxins analysed in the samples, 15 toxins were detected (> LOQ). Data on the incidence and occurrence level of individual *Fusarium* mycotoxins in the cereals (maize, sorghum, and millet) and processed products (*ogi*, *burukutu,* and *pito*) are illustrated in Tables 3.4, 3.5 and 3.6. Overall 66% prevalence rate of the mycotoxins was recorded in all sample types, with an individual rate of 77%, 44%, 59%, 97%, 78%, and 71% for maize, sorghum, millet, *ogi*, *burukutu*, and *pito*, respectively.

Maize, sorghum, millet, *ogi*, *burukutu*, and *pito* contained 13, 13, 10, 14, 11, and 8 *Fusarium* secondary metabolites, respectively, of which only five (FB_1, FB_2, DON, ZEN, and HT-2) are regulated by the European Union (EU). Fumonisins (FBs) were the most dominant mycotoxins in the cereals and *ogi*, with a much higher level and incidence rate in maize and *ogi* samples, whereas DON dominated the traditional beers (*burukutu* and *pito*). The sum of FBs (FB_1 + FB_2 + FB_3) were in the ranges of 32–8,508 µg/kg (65%), 45–180 µg/kg (8%), 74–22,064 µg/kg (14%), and 125–3,557 µg/kg (93%) in maize, sorghum, millet, and *ogi*, respectively. However, for the traditional beers, only FB_1 and FB_2 were detected at total (FB_1 + FB_2) ranges of 70–519 µg/kg (22%) and 105-245 µg/kg (18%) in *burukutu* and *pito*, respectively. Except for sorghum, some of the samples of maize (15%) and millet (1%) in this study exceeded the maximum regulatory limit set for the sum of FB_1 and FB_2 (1,000 µg/kg) by the EU (EC, 2007) suggesting a possible high exposure of consumers of these products. A similar high incidence rate of FBs has been reported in several studies from SSA [16,25–30] at concentrations ranging up to 53,863 µg/kg [27]. High incidence of FBs, especially in maize may be explained by the susceptibility of the maize crop to FBs-producing fungi (*F. verticillioides* and *F. proliferatum*) [31]. Sorghum and millet had a much lower incidence rate, however, an extreme concentration of FBs was recorded in one of the millet samples (22,064 µg/kg). Lower concentrations and incidence rate in sorghum and millet from Ethiopia have previously been reported [32]. The reversed trend was observed by Ayalew et al. [33], who recorded higher levels of FBs (range: 1,370–2,117 µg/kg) in sorghum samples. Of the FBs, FB_1 occurred at a more frequent rate than FB_2 and FB_3. Although, some of the millet (12%) and maize (5.2%) samples were observed to be contaminated with only FB_2. Such trend has previously been reported in cereals suggesting the possible contamination of *Aspergillus niger,* which is a principal producer of FB_2 [34]. The study of Ezekiel et al. [35] on sorghum grain confirms

the possible occurrence of only FB_2 in cereals from Nigeria. The incidence and levels of FBs as observed in *ogi* is of concern. The present study reveals for the first time the occurrence of *Fusarium* mycotoxins in *ogi* from the Nigerian market. The maximum concentration and percentage incidence of FB_1, FB_2, and FB_3 detected in *ogi* samples were 1,903 µg/kg (93%), 1,283 µg/kg (87%), and 371 µg/kg (77%), respectively (Table 3.5). About 83% of the *ogi* samples exceeded the EU maximum limit of 200 µg/kg for processed maize-based foods for infants and young children [24]. Interestingly, out of the 30 *ogi* samples analysed, the only two FBs negative samples were of sorghum-base. This could be linked to the reason why a low FBs incidence rate and concentration levels were observed in *burukutu* and *pito* as compared to *ogi*, as they are sorghum-based traditional beers. The trend observed in our study is in line with the previous study by Ezekiel et al. [35], who reported that sorghum is less prone to fungal infestation than maize. Although data on the occurrence of a detailed profile of *Fusarium* mycotoxins in Nigerian traditional processed food products such as *ogi* are scarce to non-existent, studies from the same country reported the occurrence of FBs in two fermented traditional cereal-based beverages (*kunu-zaki* and *pito*) [35]. In addition, $HYFB_1$ were detected in the traditional beer samples at a maximum concentration of 152 µg/kg and 102 µg/kg in 11% and 9% of *burukutu* and *pito*, respectively. Although $HYFB_1$ was initially considered as a toxin formed during alkaline processing of food, recent studies have shown its occurrence in other processed food products as well as raw cereals [16,36] (results for maize, sorghum, millet, and *ogi* are reported in Section 3.3.2). Interestingly, studies on oral administration of $HYFB_1$ in different animal species have shown that $HYFB_1$ has lesser toxic potential compared to FB_1 [37–39], thus, degradation of FBs to hydrolysed forms may be a strategy for reducing FBs exposure.

The next group of dominating mycotoxins were the TH. They have been associated with the temperate regions, however studies emerging from SSA have revealed the possible occurrence of these toxins in the tropics. Type B TH: DON, 3-ADON, 15-ADON, DON-3G, and NIV were detected in the samples of the present study. DON was the most prevalent mycotoxin in the traditional beers occurring at 65% incidence rate (range: 61-255 µg/kg) in *burukutu* and 56% (range: 65 - 184 µg/kg) in *pito* (Table 3.5). The dominance of DON in beer samples has been reported especially in European beers [10,40,41]. Abia et al. [28] also revealed the occurrence of DON (93%) in locally brewed maize beer from Cameroon with much higher values than the rate recorded in the present study. However, Matumba et al. [42] reported no DON in traditional Malawian maize-based beer, notwithstanding their study was limited to a few samples. On the other hand, DON was detected in 16%, 3%, 13%, and 13% of maize, sorghum, millet, and *ogi* samples at a maximum level of 225 µg/kg, 119 µg/kg, 583 µg/kg, and 74 µg/kg, respectively. Interestingly no sample in this study, irrespective of the food types exceeded the EU maximum limit (1,750 µg/kg, maize and 1,250 µg/kg, other

cereals) for DON [24]. The levels of DON detected in *ogi* were also below 200 µg/kg (EU limit) set for cereal-based infant foods. Incidence of DON in the cereals, as observed in this study, was similar to that reported in previous studies on Nigerian cereals [43,44], but much less than that reported in maize by Adetunji et al. [16] and Ediage et al. [30]. The same trend was reported in millet, sorghum, and cereal-based food samples from Burkina Faso [26].

Table 3.4 Mean and maximum concentration (µg/kg) of *Fusarium* mycotoxins found in cereals (maize, sorghum, and millet) from Nigeria.

Mycotoxin [1]	Maize (*n* = 136)			Sorghum (*n* = 110)			Millet (*n* = 87)		
	% + ve Samples [2]	Mean [3]	Max [4]	% + ve Samples	Mean	Max	% + ve Samples	Mean	Max
FB_1	65	541	8,222	8	64	78	9	2,333	18,172
FB_2	54	376	2,885	2	48	55	13	609	3,892
FB_3	43	117	445	2	38	46	0	na	na
∑FBs	65	935	8,508	8	83	180	14	2,113	22,064
DON	16	99	225	3	100	119	13	151	543
15-ADON	0	na [5]	na	2	39	44	1	11	11
DON-3G	0	na	na	23	24	63	0	na	na
ZEN	1	65	65	1	38	38	14	419	1,399
ZEN-14G	9	21	24	3	19	22	6	23	34
α-ZEL	1	20	20	3	33	33	0	na	na
β-ZEL	2	20	21	1	21	21	1	39	39
HT-2	1	20	20	8	20	31	5	36	36
NIV	2	206	271	0	na	na	0	na	na
FUS-X	1	154	154	0	na	na	0	na	na
DAS	13	3	8	18	5	16	29	5	25

[1] FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; ∑FBs = sum of FB_1, B_2, and B_3; DON = deoxynivalenol; 15-ADON = 15-acetyl-deoxynivalenol; DON-3G = deoxynivalenol-3-glucoside; ZEN = zearalenone; α-ZEL = α-zearalenol; β-ZEL = β-zearalenol; ZEN-14G = zearalenone-14-glucoside; NIV = nivalenol; FUS-X = fusarenon-X; HT-2 = HT-2 toxin; DAS = diacetoxyscirpenol, [2] % + = percentage positive samples, [3] Mean = mean concentrations were calculated based on the positive samples only, [4] Max = maximum concentration, [5] na = not applicable.

Table 3.5 Mean and maximum concentration (µg/kg) of *Fusarium* mycotoxins found in cereal-based products (*ogi, burukutu,* and *pito*) from Nigeria.

Mycotoxin [1]	*Ogi* (*n* = 30)			*Burukutu* (*n* = 54)			*Pito* (*n* = 45)		
	% + ve Samples [2]	Mean [3]	Max [4]	% + ve Samples	Mean	Max	% + ve Samples	Mean	Max
FB_1	93	590	1,903	22	138	316	18	114	194
FB_2	87	472	1,283	13	105	203	11	57	62
FB_3	77	121	371	0	na	na	0	na	na
∑FBs	93	1,128	3,557	22	200	519	18	150	254
$HYFB_1$	0	na [5]	na	11	95	152	9	87	102
DON	13	61	74	65	120	255	56	99	184
15-ADON	3	60	60	4	25	25	2	36	36
3-ADON	0	na	na	2	30	30	7	28	37
DON-3G	17	30	44	17	36	53	11	22	28
ZEN	3	39	39	7	41	88	4	24	25
ZEN-14G	3	31	31	2	27	27	0	na	na
α-ZEL	7	20	22	9	37	71	0	na	na
β-ZEL	10	19	20	9	87	107	0	na	na
HT-2	3	13	13	0	na	na	0	na	na
NIV	7	148	160	0	na	na	0	na	na
FUS-X	7	133	137	0	na	na	0	na	na

[1] FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; ∑FBs = sum of FB_1, B_2, and B_3; $HYFB_1$ = hydrolysed fumonisin B_1; DON = deoxynivalenol; 15-ADON = 15-acetyl-deoxynivalenol; DON-3G = deoxynivalenol-3-glucoside; ZEN = zearalenone; α-ZEL = α-zearalenol; β-ZEL = β-zearalenol; ZEN-14G = zearalenone-14-glucoside; NIV = nivalenol; FUS-X = fusarenon-X; HT-2 = HT-2 toxin; DAS = diacetoxyscirpenol, [2] % + ve = percentage positive samples, [3] Mean = mean concentrations were calculated based on the positive samples only, [4] Max = maximum concentration, [5] na = not applicable.

Studies have shown the occurrence of acetylated DON forms and DON-3G in cereals and cereal-based products [9,28]. Maize samples in our study were negative for 3-ADON, 15-ADON, and DON-3G. This is in agreement with a previous study on maize from Burkina Faso [26]. Sorghum, millet, and *ogi* were contaminated with only 15-ADON at the incidence rate of 2%, 1% and 3%, respectively, whereas 15-ADON and 3-ADON were detected in the traditional beers (*burukutu* and *pito*) (Table 3.4 and 3.5). The production of acetylated derivatives (15-ADON and 3-ADON) by *F. graminearum* have been reported and the potential of the isolates to produce 15-ADON or 3-ADON as the major isomer is dependent on the geographic origin [45,46]. Although the information on the regional relationship between *F. graminearum* and the production of 15-ADON or 3-ADON is still lacking in Africa, Li et al. [47] and Mirocha et al. [45] reported the predominance of chemotype 3-ADON in New Zealand, Austrialia, and China, while the 15-ADON chemotype is predominant in North America. DON-3G was detected in the sorghum and processed products

(*ogi*, *burukutu*, and *pito*) in the present study. A comparable result on DON-3G in sorghum and millet from Ethiopia was also reported [32]. Although no previous studies exist on the occurrence of DON-3G in Nigerian processed food products, evidence of this compound occurring in African local made beers has been reported [28]. Abia et al. [28] reported a high incidence (86%) of DON-3G in Cameroonian traditional maize-beer in contrast to the low occurrence rate observed in the present study. Samples of maize (n=3) and *ogi* (n=2) were also contaminated with NIV at concentration ranges of 163–271 µg/kg and 136–160 µg/kg, respectively. Occurrence of NIV in Nigerian maize has previously been reported, although at a higher incidence rate (54%) [16]. Castillo et al. [48] also reported the occurrence of NIV in cereal-based products similar to the rate observed in the present study. Contrary to the result reported on the occurrence of NIV in sorghum and millet by Chala et al. [32], sorghum and millet and the traditional beers (*burukutu* and *pito*) were negative for NIV in the present study. The trend observed in this study with NIV was also seen with FUS-X contamination. Also, NEO was not detected in any of the samples analysed.

With regards to type A TH, DAS and HT-2 were present in all the cereal samples, while the cereal-based products (*ogi, burukutu,* and *pito*) were negative for DAS and HT-2, except for *ogi* samples (3%) which were positive for HT-2. T-2 was negative in all the sample types. DAS was detected in maize, sorghum, and millet at a rate of 13%, 18%, and 29%, respectively (Table 3.4). The concentrations of DAS in the cereals ranged between 2 µg/kg and 25 µg/kg. The occurrence of DAS in the samples is probably associated with the occurrence of major DAS-producing fungi in this region [43,49]. DAS and HT-2 are synthesised by a wide range of *Fusarium* species, and they are alleged to be among the most toxic TH occurring in different food products. Several studies have reported the occurrence of DAS in cereals and cereal-based products [50,51]. Despite its association with the temperate weather, previous studies revealed the occurrence of DAS in tropical regions. Adejumo et al. [43] and Adetunji et al. [16] recorded the occurrence of DAS in Nigerian maize at maximum concentrations of 51 µg/kg (9%) and 30 µg/kg (19%), respectively. Besides maize, DAS has been found to contaminate sorghum and millet from Ethiopia with maximum concentrations of 64.2 µg/kg (mean value, 11.9 µg/kg), and 1.43 µg/kg (mean value, 1.43 µg/kg), respectively [32]. A total of 1%, 8%, 5%, and 3% of maize, sorghum, millet, and *ogi*, respectively, were positive of HT-2 (Table 3.4 and 3.5). Beside the low incidence rate, none of the cereals or *ogi* samples exceeded the EU recommendation level of 100 µg/kg and 15 µg/kg for cereal and infant foods, respectively [52].

Other mycotoxins detected in the study include ZEN, α-ZEL, β-ZEL, and ZEN-14G. Recent studies have shown the prevalence of ZEN in food products from SSA [32,53], however in the present study, ZEN was rarely detected. ZEN was detected in maize, sorghum, and millet at 1%, 1%, and 14%, respectively (Table 3.4) with the concentrations in all

the sample types being less than the EU maximum limit of ZEN, except for millet with 8 samples (9%) exceeding 100 µg/kg [24]. Further, only 1 (3%), 4 (7%), and 2 (4%) of *ogi, burukutu,* and *pito* samples, respectively, were positive for ZEN. While the levels of ZEN in the *burukutu* and *pito* were below the regulatory limit, the level detected in *ogi* exceeded the maximum limit of 20 µg/kg set by EU for processed cereal-based foods for infants and young children [24]. About 1% and 2% of maize were contaminated with α-ZEL and β-ZEL, respectively. Although there exist only limited studies on the occurrence of these metabolites in food products from SSA, available data show their possible occurrence in Nigerian maize [16,53]. The present study is in agreement with the result of Adetunji et al. [16]. The maximum levels for β-ZEL in sorghum and millet were 21 µg/kg and 39 µg/kg, respectively. Millet samples were negative of α-ZEL, while sorghum had a 3% incidence rate with maximum levels of 33 µg/kg (Table 3.4). Chala et al. [32] reported a higher incidence rate of α-ZEL and β-ZEL in sorghum and millet [32] compared to the current study, however, the levels reported by these authors were lower. α-ZEL and β-ZEL were detected in *ogi* and *burukutu* samples, while a reverse trend was observed with the *pito* samples (Table 3.5). ZEN metabolites have been previously reported in traditional brewed beers [28,35]. However, the occurrence rate reported by Abia et al. [28] was much higher (α-ZEL - 86% and β-ZEL - 93%) than observed in the present study. With regards to ZEN-14G, sample of maize (9%), sorghum (3%), millet (6%), *ogi* (3%), and *burukutu* (2%) were positive, with maximum concentrations of 24 µg/kg (maize), 22 µg/kg (sorghum), 34 µg/kg (millet), 31 µg/kg (*ogi*), and 27 µg/kg (*burukutu*). Occurrence of this modified form of ZEN in cereals and cereal-based products (*ogi* and *burukutu*) in the current study is supported by a study which detected a wide range of modified forms of ZEN in cereal-based food products [13,54]. Although, there is no recommendation nor maximum limit for ZEN-14G in cereals and cereal-based products because of the non-availability of toxicological data, the occurrence of ZEN-14G as observed in the present study is presumed to add additional toxic effect to the host. De Boevre et al. [55] and Dall'Asta et al. [56] reported the possible hydrolysis of ZEN-14G and modified fumonisins into their free forms: ZEN and FBs, respectively in the digestive tract of mammals suggesting an additional toxicity.

3.3.2. Fumonisin and modified fumonisin contamination in cereals (maize, sorghum, and millet) and processed products (*ogi*) from Nigeria

To determine the occurrence of modified fumonisins, samples were selected from each food type based on the FBs result obtained from the multi-mycotoxin analysis (Table 3.4 and 3.5). Five FBs positive and 5 FBs negative samples of each food type (maize, sorghum, and millet) were selected for analysis. Note that 8 positive samples and 2

negative samples of *ogi* were used for the analysis, because only two samples of *ogi* were negative. Each sample was analysed simultaneously for FBs (FB_1, FB_2, and FB_3), as well as total fumonisins after hydrolysis as described in Section 3.2.5. Calculation of modified FBs concentration was based on the difference between the concentration of FBs and the concentration of total FBs after hydrolysis [57]. The results are depicted in Figure 3.3, Table 3.6 and Annex 3.7 (result for individual samples). The maximum concentration of free FBs and total FBs (after hydrolysis) in the selected samples of maize, sorghum, millet, and *ogi* were 3,514 and 4,568 µg/kg, 180 and 502 µg/kg, 840 and 3,059 µg/kg, and 1,496 and 1,795 µg/kg, respectively. After hydrolysis, an increment was observed ranging from 1.3 to 5.2 times higher levels of total FBs in maize samples. The same trend was observed in sorghum, millet, and *ogi* samples.

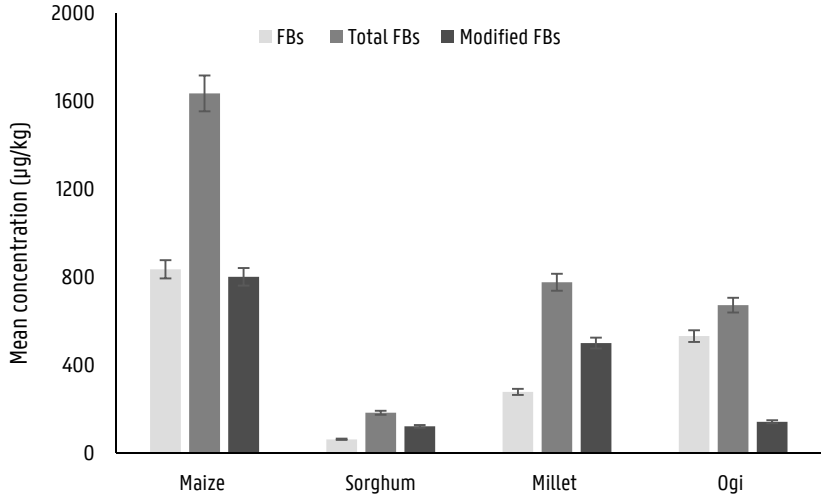

Figure 3.3. Mean concentration of free and modified fumonisins in different food types: maize, sorghum, millet, and *ogi*

Table 3.6 Contamination levels of fumonisins, total fumonisins, and modified fumonisins in selected samples.

Food Type	FBs (µg/kg)			Total FBs (µg/kg)			Modified FBs (µg/kg)		
	Median	Mean	Maximum	Median	Mean	Maximum	Median	Mean	Maximum
Maize (*n* = 10)	358	835	3,514	543	1,636	4,568	144	801	2,923
Sorghum (*n* = 10)	41	61	180	95	182	502	50	120	323
Millet (*n* = 10)	118	277	840	302	776	3,059	179	499	2,254
Ogi (*n* = 10)	247	531	1,496	391	672	1,795	117	141	313

FBs = free fumonisins, total FBs = total fumonisins detected after hydrolysis, modified FBs concentration = the difference between the concentration of FBs and the concentration of total FBs after hydrolysis.

Modified FBs have been alleged to occur in processed products, especially nixtamalised and thermally processed foods [57,58]. However, recent studies have revealed the occurrence of these toxins in unprocessed food products especially in raw maize samples [56,59], which suggest the possible transformation of FBs to bound derivatives by natural phenomena due to plant metabolism [20]. The presence of modified FBs as observed in the current study may pose an additional health risk to consumers, especially to the consumers of *ogi* analysed in this study. FBs have been alleged to cause a range of toxic health effects on humans and animals, especially in SSA where cases of very high levels of FBs have been recorded. Cases of human oesophageal cancer in South Africa and other parts of the world have been linked to the consumption of food contaminated with FBs. Since it is obvious that modified FBs may cause an additional toxic effect on the host as observed when low FBs contaminated feed was fed to animals [60], the occurrence of modified FBs in cereals and cereal-based products should no longer be neglected especially in Nigeria where these products serve as major staple food.

3.3.3 Distribution of *Fusarium* mycotoxins in major cereals across the different agro-ecological zones of Nigeria

Mycotoxin occurrence and distribution is influenced by different factors including crop species, climatic, and environmental conditions of a given region. The mean and maximum concentrations of individual mycotoxins in the different food types and AEZs are shown in Table 3.7. Fumonisin contaminations were observed in all the cereal types irrespective of the AEZ (Figure 3.4). Sudan Savanna (SS) and Northern Guinea Savanna (NGS) zones had the highest incidence rate of FB_1 in maize with a highest FB_1 concentration of 2,443 µg/kg and 8,222 µg/kg, respectively, when compared to Southern Guinea Savanna (SGS) and Derived Savanna (DS). A similar trend with FB_1 contamination was seen when the sum of FB (FB_1, FB_2, and FB_3) was considered. This is also similar with the result obtained from the sorghum samples, with the SS zone registering the highest FB_1 followed by NGS and SGS. This observation could be linked to the high mycotoxin production potentials of *Fusarium* fungi in warmer climates [61] and the significant change in climatic conditions in this region characterised by an increase in rainfall and longer raining seasons [62]. Among the other mycotoxins detected, DAS was the next most common metabolite contaminating all the cereal types across the AEZs, although at lower concentrations. While there are no existing regulatory limits set for DAS in food products, DAS has been implicated in a wide range of toxic effects in animals as well as humans, ranging from acute to chronic. It has been linked to a human fatal disease (alimentary toxic aleukia), exhibiting several symptoms such as inflammation of the skin, vomiting, and damage to hematopoietic tissues [63,64]. DON also occurred in the respective cereal types across the AEZs, except for the sorghum samples from the SS zone (Table 3.7).

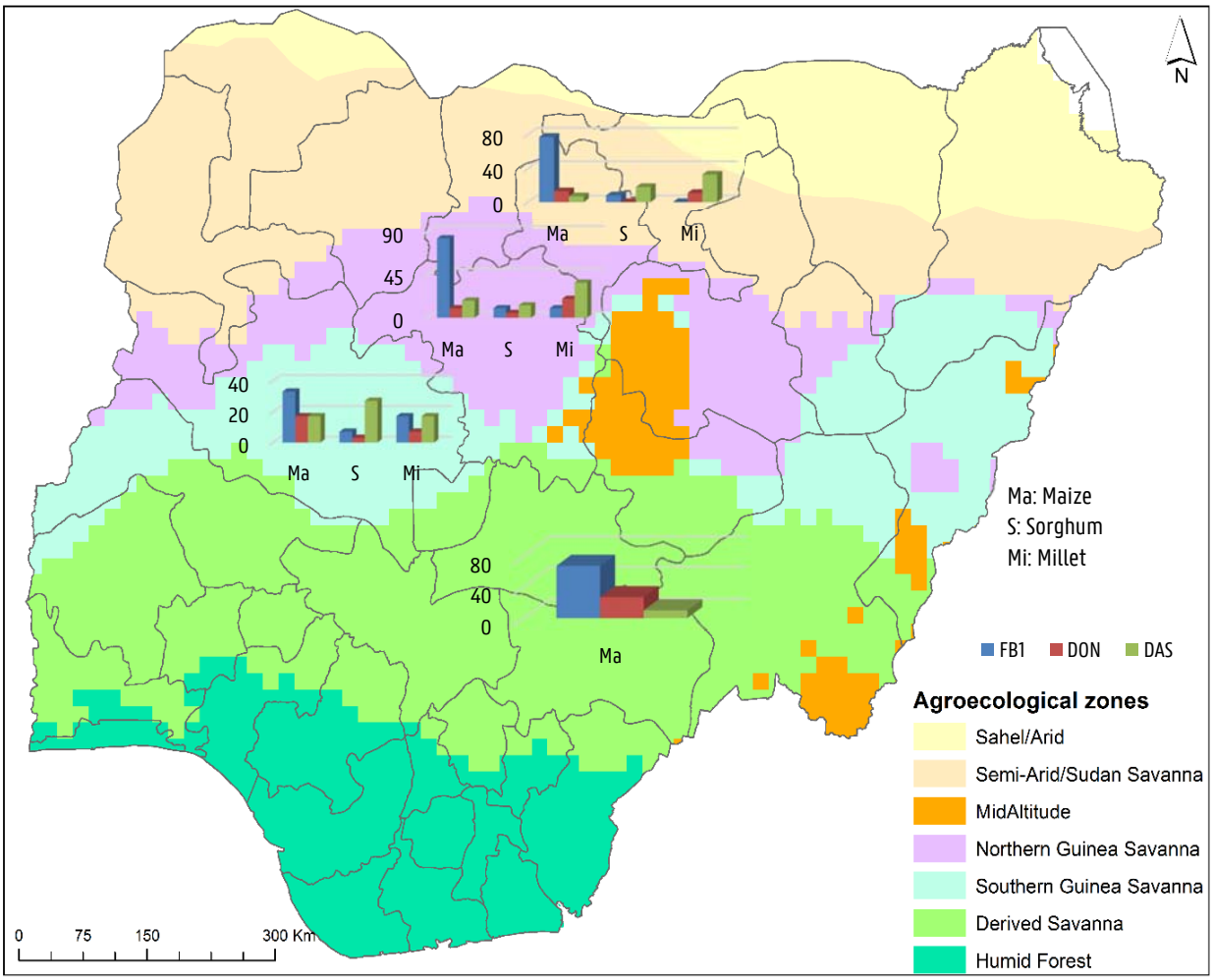

Figure 3.4. Percentage incidence rate of *Fusarium* mycotoxins in Nigerian cereals (maize, sorghum, and millet) from different agro-ecological zones. FB_1 = fumonisin B_1, DON = deoxynivalenol, DAS = diacetoxyscirpenol

The highest incidence rate of DON was observed in maize samples from the DS zone characterized by a lower temperature and higher average annual rainfall of 25–35 °C and 1300–1500 mm, respectively, when compared to the other zones. In millet samples, the NGS zone registered the highest incidence rate of DON. Comparing the incidence of ZEN across the different food types and AEZs, samples from SS zones were negative of ZEN regardless of the cereal type. This result could be related to the prevailing local weather conditions of this region which is between 30 and 40 °C, which is above the optimum temperature of 25 °C for the production of ZEN [65].

Table 3.7. *Fusarium* mycotoxins occurrence in cereals (maize, sorghum, and millet) across the different agro-ecological zones of Nigeria

Mycotoxin [1]	Maize (µg/kg)								Sorghum (µg/kg)				Millet (µg/kg)							
	DS (*n* = 30)		SGS (*n* = 36)		NGS (*n* = 40)		SS (*n* = 30)		SGS (*n* = 30)		NGS (*n* = 40)		SS (*n* = 40)		SGS (*n* = 30)		NGS (*n* = 30)		SS (*n* = 27)	
	Mean [2] (% + ve) [3]	Max [4]	Mean (% + ve)	Max	Mean (% + ve)	Max	Mean (% + ve)	Max	Mean (% + ve)	Max	Mean (% + ve)	Max	Mean (% + ve)	Max	Mean (% + ve)	Max	Mean (% + ve)	Max	Mean (% + ve)	Max
FB_1	117 (67)	366	249 (33)	876	928 (83)	8,222	505 (77)	2,443	70 (7)	71	59 (10)	76	67 (8)	78	3,700 (17)	18,172	54 (10)	84	na	na
FB_2	289 (57)	1,011	350 (28)	677	508 (65)	2,885	295 (70)	1,107	na	na	41 (3)	41	55 (3)	55	417 (37)	3,892	56 (13)	103	44 (7)	47
FB_3	114 (47)	353	147 (25)	445	126 (53)	441	91 (50)	213	na	na	31 (3)	31	46 (3)	46	na	na	na	na	na	na
DON	78 (27)	147	99 (17)	180	98 (10)	151	140 (13)	225	119 (3)	119	91 (5)	92	na	na	140 (7)	200	171 (20)	543	118 (11)	118
15-ADON	na [5]	na	na	na	na	na	na	na	34 (3)	34	44 (3)	44	na	na	na	na	na	na	11 (4)	11
DON-3G	na	na	na	na	na	na	na	na	12 (27)	16	30 (40)	63	22 (3)	22	na	na	na	na	na	na
ZEN	na	na	na	na	65 (3)	65	na	na	38 (3)	38	na	na	na	na	481 (33)	1,399	109 (7)	198	na	na
ZEN-14G	20 (23)	24	21 (6)	22	23 (8)	23	na	na	20 (3)	20	19 (5)	22	na	na	29 (7)	34	19(7)	20	23 (4)	23
α-ZEL	na	na	20 (3)	20	na	na	na	na	na	na	33 (8)	33	na	na	na	na	na	na	na	na
β-ZEL	20 (3)	20	na	na	21 (3)	21	na	na	21 (3)	21	na	na	na	na	na	na	39	39	na	na
HT-2	20 (3)	20	na	na	na	na	na	na	19 (17)	19	24 (8)	31	11 (3)	11	35 (7)	35	36 (7)	36	na	na
NIV	228 (7)	271	na	na	na	na	163 (3)	163	na	na	na	na	na	na	na	na	na	na	na	na
FUS-X	154 (3)	154	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na	na
DAS	2 (10)	2	3 (17)	6	3 (18)	4	8 (7)	8	5 (27)	13	4 (13)	5	5 (18)	16	12 (17)	25	4 (37)	6	3 (33)	4

DS = Derived Savanna; SGS = Southern Guinea Savanna; NGS = Northern Guinea Savanna; SS = Sudan Savanna. [1] FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; DON = deoxynivalenol; 15ADON = 15-acetyl-deoxynivalenol; DON-3G = deoxynivalenol-3-glucoside; ZEN = zearalenone; α-ZEL = α-zearalenol; β-ZEL = β-zearalenol; ZEN-14G = zearalenone-14-glucoside; NIV = nivalenol; FUS-X = fusarenon-X; HT-2 = HT-2 toxin; DAS = diacetoxyscirpenol; [2] Mean = mean concentrations were calculated based on positive samples, [3] % + ve = percentage positive samples, [4] Max = maximum concentration, [5] na = not applicable.

In general, it is postulated that *Fusarium* fungi and subsequent mycotoxins occurrence is higher in colder regions. A trend in the incidence of *Fusarium* toxins was observed in this study, with approximately colder region having multiple mycotoxins (Table 3.7). Further analysis to assess the significant difference in the distribution of *Fusarium* mycotoxins across the AEZs was done using the Kruskal Wallis test (Figure 3.5). With respect to maize, there was a significant difference in FB_1 and DAS contamination across the AEZs, whereas in millet samples, DAS contamination was significantly different across the different zones. For the other *Fusarium* mycotoxins detected (FB_2, FB_3, DON, 15-ADON, DON-3G, ZEN, α-ZEL, β-ZEL, ZEN-14G, NIV, FUS-X, HT-2) in the cereal samples, there was no significant difference was observed across the AEZs. This result may be attributed to the quality of the sampled food products. This is because of the premium placed on high quality (visual observation) food products which arises through sorting and cleaning in order to add more value to the products, thus increasing the market value of the food product

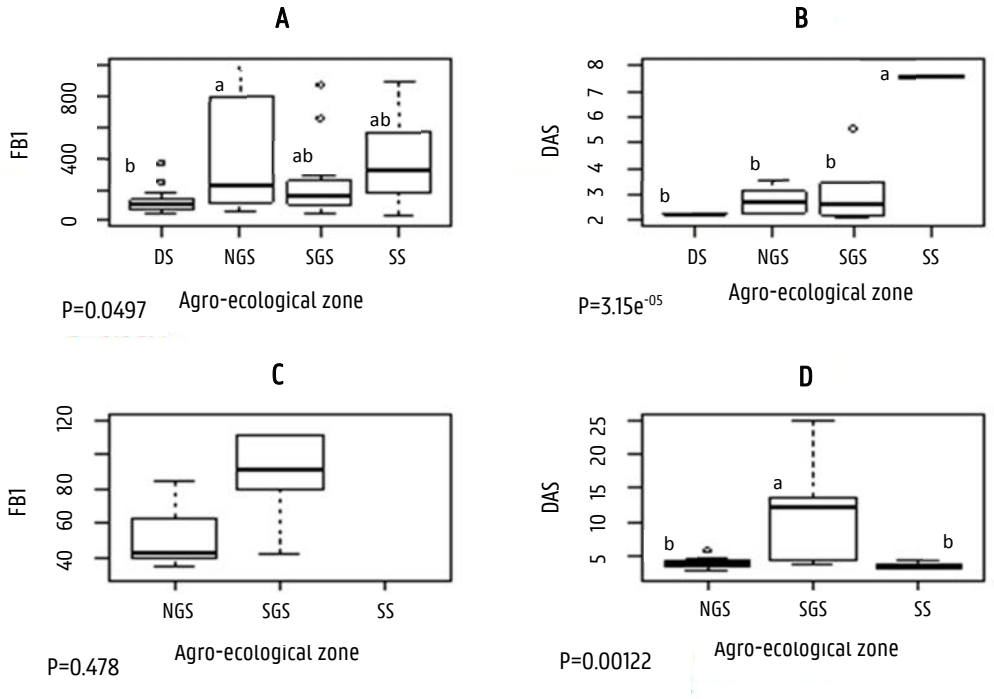

Figure 3.5. Differences in *Fusarium* mycotoxins in maize ((A) fumonisin B_1; and (B) diacetoxyscirpenol); and millet ((C) fumonisin B_1; and (D) diacetoxyscirpenol) across agro-ecological zones. Agro-ecological zones with the same lower-case letter are not significantly different (P≤0.05) according to Kruskal Wallis test

3.3.4 Co-occurrence of *Fusarium* mycotoxins in cereals and processed products (*ogi, burukutu, and pito*) from Nigeria

Occurrence of multiple mycotoxins in food, especially cereals and cereal-based products has been an issue of great concern because of the synergistic and/or additive effects caused by the interaction of these toxins in humans and animals. In this study, we observed that 60%, 19%, 30%, 93%, 41%, and 36% of maize, sorghum, millet, *ogi, burukutu,* and *pito,* respectively, were contaminated with at least two mycotoxins (Figure 3.6). In maize samples, FBs co-existed with DAS at 13%, being the highest level observed, followed by co-occurrence between FBs and DON (11%). DAS and DON-3G co-occurred in 16% of sorghum samples, succeeded by DON-3G and HT2, while DAS and ZEN dominated in millet samples followed by DAS and DON. Previous studies have shown the frequent co-contamination of *Fusarium* toxins in cereal products in SSA [16,28,30,32], which is linked to co-occurrence of several species of *Fusarium* fungi in crops or the potential ability of one *Fusarium* spp. to produce more than one mycotoxin [66]. However, the rates observed in sorghum and millet in the present study were in contrast with the data reported on co-occurrence of mycotoxins in sorghum (94%) and millet (85%) from South Korean retail markets [67], and may be attributed to the different sampling regions considered for these studies.

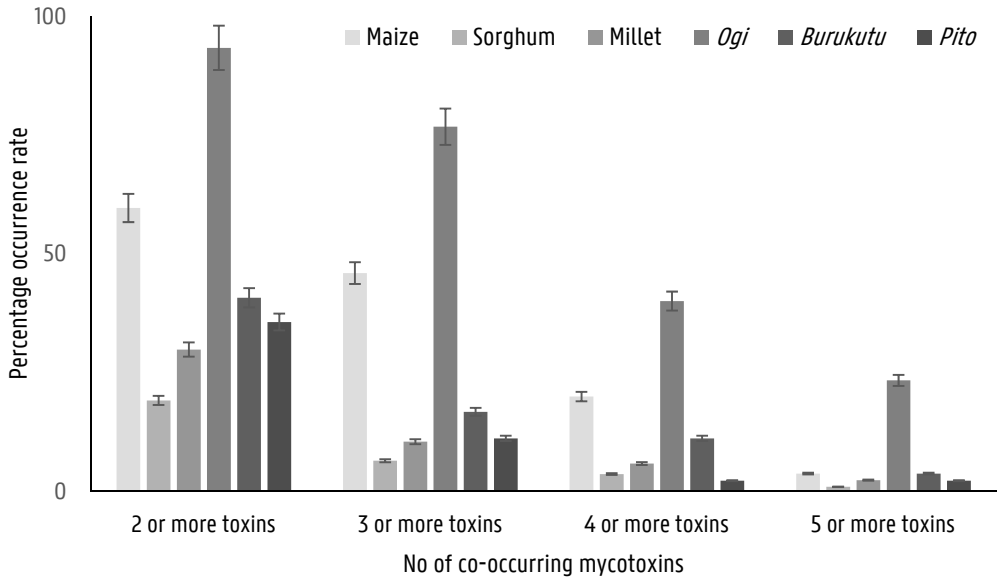

Figure 3.6 Percentage of co-occurrence of *Fusarium* mycotoxins in cereals (maize, sorghum, and millet) and cereal-based products (*ogi, burukutu,* and *pito*) from Nigerian markets.

Although only limited studies exist on the co-occurrence of DON, ZEN, and their modified forms in SSA, available data proves the potential of DON co-occurring with its modified forms in cereals from this region [30]. This was not the case in this study. The disparity may be attributed to the differences in sampling protocol. Ediage et al. analysed household samples [30], while samples from the markets were analysed in the present study. Cereals purchased from the market often are of better quality compared to those from the household or at farmers' disposal. Ironically, this is because high-graded cereal grains are often placed for sale by the farmers because of a better market bargain, while they retain the poor-quality cereal grains since there are no provisional channels for destruction or diversion of these products.

Interestingly, the processed cereal-based products (*ogi, burukutu,* and *pito*) analysed in this study had the highest co-occurrence rate compared to the cereals except for maize which exceeded *burukutu*. This observation could be attributed to many factors (such as poor sanitation during processing). *Ogi, burukutu,* and *pito* are fermented under uncontrolled conditions, and as such could be contaminated with any form of organism residing in the environment. Another possible avenue for contamination is the quality of the raw cereal used in the production of these products. Although reduction of mycotoxin concentrations have been reported during processing of *ogi* [2] and *pito* [35], it is important to mention that reduction of mycotoxins during food processing is dependent on the initial concentrations of the raw produce and as such good quality cereal grains should be used for production of food products. Furthermore, the rate of co-occurrence observed in *ogi* exceeded that of the traditional beers (*burukutu* and *pito*). While 4% and 2% of *brurukutu* and *pito*, respectively, were contaminated with at least 5 mycotoxins, approximately 23% of *ogi* samples were contaminated with at least 5 mycotoxins. A similar trend was revealed by Ezekiel et al. [35], who reported a higher contamination rate in maize-based products compared to sorghum-based products. The highest co-occurrence rate in *ogi* existed between FBs and DON-3G, and subsequently between FBs and DON. DON and DON-3G co-occurred in 4 of the samples while ZEN, ZEN-14G, α-ZEL, and β-ZEL co-occurred in one sample. For the traditional beers, DON and FBs had the highest co-occurrence rate of 19% (*burukutu*) and 9% (*pito*), followed by DON and DON-3G (11% - *burukutu* and 7% - *pito*). This result is affirmed by the previous study on co-occurrence of these metabolites in cereal-based products [9]. However, the rates and concentration levels in their study were higher than the levels observed in this study.

3.4 Conclusions

The result from this study showed the occurrence of *Fusarium* toxins in cereals and cereal-based fermented products (*ogi, burukutu,* and *pito*) from Nigeria. It revealed for the first-time the occurrence of *Fusarium* mycotoxins in *ogi* from the Nigerian markets. FBs were the most dominating *Fusarium* mycotoxins in the cereals and *ogi* samples with some of the samples exceeding the FBs regulatory limits set by the EU. DON was prevalent in traditional beers. Although the levels of other mycotoxins detected in the samples were low, the co-occurrence of these mycotoxins presents a health risk due to the synergistic and/or additive effect (Chapter 1), considering the fact that cereals and cereal-based products are consumed almost on daily basis. Except for FB_1 and DAS, there were no significant difference in the occurrence of mycotoxins in cereals from Nigerian markets across the AEZ. This could be attributed to pre-treatment such as sorting and cleaning given to these grains prior to marketing, however there is a need for continues monitoring. In addition, it is worrisome that the traditional weaning food, fed to a large population of infants and growing children in Nigeria contains high levels of FBs with a cocktail of other mycotoxins. This result shows that infants and young children may be affected with different variation of toxic effects. Reducing mycotoxins in processed food products is critical. Relentless efforts should be channelled towards rural and urban level advocacy on the need to use quality seeds of maize, millet, or sorghum as raw materials for processing. Within this context, it is imperative and timely to educate the small-scale producers on the risk of mycotoxins and possible ways to reduce or avoid contamination. The occurrence of *Fusarium* mycotoxins in some other Nigerian food commodities are presented subsequently (Chapter 4 and 5).

3.5 References

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PREVALENCE OF *FUSARIUM* MYCOTOXINS IN ROOTS AND TUBER DERIVED PRODUCTS FROM NIGERIAN MARKETS

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CHAPTER 4: PREVALENCE OF *FUSARIUM* MYCOTOXINS IN ROOTS AND TUBER DERIVED PRODUCTS FROM NIGERIAN MARKETS

4.1 Introduction

Roots and tubers serve as major staple foods in the developing countries especially among the rural poor population in Africa, thus contributing substantially to food security. Among the roots and tubers, cassava (*Manihot esculenta*) and yam (*Dioscorea* spp.) are ranked as the most important staple foods in sub-Saharan Africa. The African continent contributes about 53% and 96% of the world's production, respectively [1]. Nigeria maintains the first in the world's production ranking of both crops with about 46 million tonnes and 37 million tonnes of cassava and yam produce annually, respectively. It is estimated that an average adult, especially in West Africa, consumes about 80 kg and 61 kg per capita of cassava and yam per annum, respectively. Furthermore, cassava and yam account for a high source of energy intake of a large population of Nigerians. These crops are constrained because of their high deterioration rate usually caused by microbial activities. They are often processed into several food forms, such as fermented cassava flour (*lafun*) and fermented yam flour (*amala*) to increase the shelf-life and create more food varieties in the region [2,3]. Additionally cassava can be processed into *garri*, *abacha*, *fufu,* and contributes a large percentage in the formulation of animal feeds and raw materials for industries [3,4].

Garri is a dry granular product processed by dewatering and fermentation of peeled cassava tubers and subsequent granulating and garification in a hot frying tray or pan. The processing of *lafun* and *amala* involves peeling and slicing of cassava and yam tubers, respectively, and subsequently steeping/fermenting and sun-drying for 5 to 7 days depending on the climatic conditions. However, these products are exposed to various environmental contamination sources such as insects and fungi infestation because of the rudimentary techniques and uncontrolled poor drying conditions used for processing. The fermentation of *garri, lafun*, and *amala* is predominantly by *Lactobacillus* spp., *Bacillus* spp., *Staphylococcus,* and *Streptococcus* spp. [5,6]. Other microorganisms that have been isolated include yeast, *Escherichia* spp., *Micrococcus* spp., *Leuconostoc* spp., *Pseudomonas* spp., and *Corynebacterium* spp. [5–7]. Evidence of contamination of cassava and yam products with pathogenic fungi has been documented in several studies [8,9] suggesting possible occurrence of their secondary metabolites (mycotoxins) in the products. Ediage et al. [10], Bassa et al. [11], and Makun et al. [12] also reported the occurrence of toxigenic fungi and mycotoxins especially those belonging to *Aspergillus* species. A recent study by Nyaka et al. [13] also revealed the occurrence of other toxigenic fungi such as the *Fusarium* spp. as a causative agent

of cassava root rot disease, and this corroborated with the study of Manjula et al. [14], who detected FBs in cassava products.

The toxic effects due to human and animal exposure to *Fusarium* mycotoxins has been a subject of discourse since the last decade (Chapter 1). In spite of the toxic effects caused by these *Fusarium* mycotoxins and the high propensity of these products to contamination during processing and storage, several authors have documented only the problems associated with *Aspergillus* mycotoxins [12,15], thus little or no information is known on the occurrence of *Fusarium* mycotoxins in these products. Therefore, this study reports for the first-time a detailed profile of *Fusarium* mycotoxins in cassava (*garri, lafun*) and yam (*amala*) products sold in Nigerian markets.

4.2 Materials and methods

4.2.1. Sample collection

A total of 94 samples comprising of cassava products (*garri*, n=24 and *lafun*, n=36) and yam products (*amala*, n=34) were collected between September 2015 and October 2015 for this study. The areas of sampling were selected based on the major producing areas of the food products. *Lafun* and *amala* were sampled from randomly selected markets in Ogun state and Ekiti state in DS zone, Nigeria, while *garri* samples were collected from markets in Anambra state in HF zone, Nigeria. After collection, each sample was thoroughly homogenised, and a representative portion of 200 g was taken, and packed into a transparent zip lock bag, and transported to the Laboratory of Food Analysis (Ghent University, Belgium) for further analysis. Prior to analysis, each sample was thoroughly homogenised, blended for 1 min using an IKA M20 universal mill (Sigma-Aldrich, Bornem, Belgium) and stored at -20 °C.

4.2.2 Chemicals and Reagents

All the chemicals and reagents used for sample preparation and LC-MS/MS analysis were of analytical grade. Their sources and preparations are the same as described in Chapter 3 (Section 3.2.2).

4.2.3. Mycotoxin Analysis

The mycotoxin analysis of the cassava (*lafun* and *garri*) and yam (*amala*) products were performed using an LC-MS/MS method for multi-mycotoxin analysis by Monbaliu et al. [16] as described in Chapter 3 (Section 3.2.3). The

method was validated for cassava (*lafun* and *garri*) and yam (*amala*) products (as described in Section 3.2.7) considering the following validation parameters: apparent recovery, LOD, LOQ, repeatability, and reproducibility. The data on the method performance characteristics including the LOD, LOQ, and apparent recovery of the food matrices are presented in Table 4.1. While the results of the Intraday repeatability and interday reproducibility of the individual mycotoxins for cassava (*Garri*, *Lafun*) and yam products (*Amala)* are presented in Table 4.2.

Table 4.1. LC-MS/MS method performance characteristics of cassava (*Garri*, *Lafun*) and yam products (*Amala)*

	LOD (µg/kg)		LOQ (µg/kg)		Apparent recovery (%)	
Mycotoxin	*Garri/Lafun*	*Amala*	*Garri/Lafun*	*Amala*	*Garri/Lafun*	*Amala*
DON	14.5	18.2	29.0	36.4	100.0	93.7
3-ADON	11.8	11.5	23.5	23.0	95.9	96.8
15-ADON	8.5	4.8	17.0	9.5	94.9	97.8
DON-3G	3.2	2.8	6.3	5.6	89.9	98.1
FB_1	15.0	12.6	30.0	25.2	71.8	96.1
FB_2	10.5	13.7	21.1	27.3	91.5	95.8
FB_3	19.5	16.0	38.9	32.0	94.5	78.0
ZEN	3.6	3.8	7.2	7.6	90.5	94.0
α- ZEL	4.3	4.3	8.6	8.6	92.0	96.0
β-ZEL	5.2	5.2	10.4	10.4	86.2	93.2
ZEN-14G	6.8	6.8	13.6	13.6	84.0	82.0
DAS	2.0	1.4	4.0	2.8	88.0	92.6
NEO	5.6	3.9	11.2	7.8	92.0	100.2
T-2	4.5	3.4	9.1	6.8	87.8	92.7
HT-2	5.4	4.6	10.8	9.1	89.2	98.5
FUS-X	54.5	58.4	108.0	116.8	92.1	97.1
NIV	57.0	44.3	114.0	88.6	104.0	99.7

LOD = limit of detection, LOQ = limit of quantification, DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, DAS = diacetoxyscirpenol, NEO = neosolaniol, T-2 = T-2 toxin, HT-2 = HT-2 toxin, FUS-X = fusarenon-X, NIV = nivalenol.

Table 4.2 Intraday repeatability (RSD_r) and interday reproducibility (within-laboratory) (RSD_R) of the individual mycotoxins for cassava (*Garri*, *Lafun*) and yam products (*Amala)*

	RSD_r (%)						RSD_R (%)					
Mycotoxin	Conc. (µg/kg)	*Garri/Lafun*	*Amala*	Conc. (µg/kg)	*Garri/Lafun*	*Amala*	Conc. (µg/kg)	*Garri/Lafun*	*Amala*	Conc. (µg/kg)	*Garri/Lafun*	*Amala*
FB_1	200	8.9	9.1	800	12.1	8.2	200	8.8	14.0	800	7.5	7.0
FB_2	200	12.8	8.6	800	8.7	5.0	200	19.2	15.0	800	12.0	8.0
FB_3	25	13.6	10.1	100	11.6	7.7	25	13.1	13.9	100	10.1	11.5
DON	200	12.6	8.4	800	9.7	8.0	200	20.5	20.1	800	14.1	13.7
3-ADON	25	6.5	10.2	100	11.4	20.1	25	8.3	7.8	100	12.4	7.3
15-ADON	12.5	9.0	12.1	50	7.8	16.3	12.5	13.3	11.1	50	14.2	16.7
DON-3G	5	12.2	17.5	20	9.5	13.6	5	18.4	9.3	20	13.2	7.4
ZEN	50	6.3	18.6	200	8.6	11.2	50	14.3	12.1	200	19.1	9.3
α- ZEL	50	11.1	10.4	200	10.0	9.4	50	13.2	13.1	200	13.7	12.1
β-ZEN	50	13.0	11.1	200	11.2	9.7	50	14.1	18.1	200	10.0	10.0
ZEN-14G	50	15.5	16.1	200	5.6	13.0	50	16.5	13.5	200	13.5	18.5
NIV	100	11.4	13.0	400	9.4	9.4	100	13.4	19.1	400	14.5	13.1
FUS-X	100	10.5	8.0	400	8.6	7.4	100	8.3	10.1	400	9.3	15.4
T-2	50	10.6	15.4	200	8.5	13.6	50	8.3	8.9	200	14.0	13.2
HT-2	50	12.3	16.7	200	10.1	14.0	50	19.1	15.4	200	7.4	14.3
DAS	2.5	9.1	11.3	10	8.2	9.4	2.5	11.3	12.6	10	13.4	16.0
NEO	50	10.7	10.0	200	7.5	9.7	50	20.0	15.0	200	15.2	16.2

RSDr = Intraday repeatability, RSDR = interday reproducibility (within-laboratory), FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3, DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, NIV = nivalenol, FUS-X = fusarenon-X, T-2 = T-2 toxin, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, NEO = neosolaniol.

4.3 Results and discussion

This study reports for the first time a wide range of *Fusarium* mycotoxins in cassava (*garri* and *lafun*) and yam (*amala*) products from Nigerian markets. Overall, the different food products were contaminated with at least 7 different *Fusarium* mycotoxins including DON, 3-ADON, 15-ADON, DON-3G, FB_1, FB_2, FB_3, ZEN, DAS, T-2, and FUS-X (Table 4.3). Previous studies have reported the incidence of mycotoxins (ochratoxin A and aflatoxins) in cassava and yam products from Nigerian markets [12,17]. Evidence of occurrence of *Fusarium* spp. in yam and cassava products have also been reported by several authors [18–20] which suggest possible occurrence of *Fusarium* mycotoxins in the products as observed in the present study. The occurrence of mycotoxins in these products may be attributed to the rudimentary processing, poor storage, and marketing practices used for these products. A total of 54%, 72%, and 82% of *garri, lafun*, and *amala* samples, respectively, were contaminated with at least one of these mycotoxins. Furthermore, 4 of the EU regulated mycotoxins (DON, FB_1, FB_2, ZEN) and DAS occurred in all the sample types.

FB_2 was the most prevalent *Fusarium* mycotoxin based on an incidence rate of 53% in the total samples, with a concentration range of 29-65 µg/kg (mean: 40 µg/kg), 30-392 µg/kg (mean: 116 µg/kg), and 29-155 µg/kg (mean: 77 µg/kg) in *garri, lafun,* and *amala* samples, respectively (Table 4.3). The occurrence of only FB_2 in cereal samples from Nigeria has also been reported by Chilaka et al. [21] and Ezekiel et al. [22]. The prevalence of FB_2 in this study suggests possible contamination of the samples with *Aspergillus niger* which is a major producer of FB_2 [23,24]. Previous mycological studies in this region reported the occurrence of *A. niger* in cassava and yam products [18,25]. Gnonlonfin et al. [26] and Onana et al. [27] also reported *A. niger* as one of the dominant fungal spp. contaminating cassava products in Benin and Cameroon. FB_1 was predominant in *lafun* samples at an incidence rate and mean concentration of 44% and 110 µg/kg, respectively, while a quarter of the *garri* samples were contaminated with FB_1 at a mean concentration of 60 µg/kg. *Amala* samples had the least incidence rate of FB_1 (21%) at a concentration range of 36 µg/kg to 124 µg/kg. Of the food products analysed, FB_3 was only present in *amala* samples ranging between 38 µg/kg and 42 µg/kg. The occurrence of FBs in yam and cassava products had previously been reported [14,17]. Somorin et al. [17] also reported a high value of 91 µg/kg of FB_1 in Nigerian yam products (*amala*), while Manjula et al. [14] reported a value of 320 µg/kg in cassava products sampled from markets in Tanzania and The Republic of the Congo. Onana et al. [27] reported the occurrence of *F. verticillioides,* a major producer of FBs in cassava chips from Cameroon.

Table 4.3 Occurrence and levels of *Fusarium* mycotoxins (µg/kg) in cassava (*garri*, *lafun*) and yam (*amala*) products from some Nigerian markets

		Concentration of positive samples										
		DON	3-ADON	15-ADON	DON-3G	FB_1	FB_2	FB_3	ZEN	DAS	T-2	FUS-X
	% + ve sample	38	0	0	13	25	21	0	17	8.3	13	0
Garri	Mean (µg/kg)	57	na	na	16	60	40	na	14	7.5	19	na
(n=24)	Std dev (µg/kg)	19	na	na	4.5	13	15	na	2.5	3.0	2.6	na
	Max (µg/kg)	99	na	na	20	80	65	na	17	9.6	22	na
	Min (µg/kg)	35	na	na	12	45	29	na	11	5.4	17	na
	% + ve sample	29	0	8.3	0	44	61	0	5.6	31	0	8.3
Lafun	Mean (µg/kg)	62	na	30	na	110	116	na	15	14	na	143
(n=36)	Std dev (µg/kg)	19	na	7.6	na	71	89	na	1.7	5.7	na	16
	Max (µg/kg)	91	na	36	na	256	392	na	16	22	na	159
	Min (µg/kg)	31	na	21	na	44	30	na	13	6.7	na	128
	% + ve sample	41	12	5.9	0	21	68	5.9	8.8	27	2.9	2.9
Amala	Mean (µg/kg)	68	31	14	na	71	77	40	15	7.8	8.6	135
(n=34)	Std dev (µg/kg)	26	3.6	0.2	na	33	38	2.8	4.7	6.1	na	na
	Max (µg/kg)	125	35	14	na	124	155	42	19	21	8.6	135
	Min (µg/kg)	39	27	14	na	36	29	38	9.4	2.9	8.6	135

% + ve sample= percentage positive samples, mean = mean concentrations were calculated based on the positive samples only, Std dev = standard deviation, Max = maximum concentration, Min = minimum concentration, na = not applicable, DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3, ZEN = zearalenone, DAS = diacetoxyscirpenol, T-2 = T-2 toxin, FUS-X = fusarenon-X.

Among the type A TH quantified, DAS was positive in all the sample types at a mean concentration and incidence rate of 8 µg/kg (8%), 14 µg/kg (31%), and 8 µg/kg (27%) for *garri*, *lafun*, and *amala*, respectively (Table 4.3). Occurrence of T-2 was observed in 13% and 3% of *garri* and *amala* samples at low concentration levels (*garri*, max: 22 µg/kg and *amala*, max: 9 µg/kg), respectively. Though occurrence of DAS and T-2 has not been previously reported in these products, earlier studies recorded the potential occurrence of DAS and T-2 in food products (cereals) from Nigerian markets [21,28]. Though there are no regulatory limits for DAS, clinical studies showed its high toxic potency in different animal species [29]. The levels of T-2 in the samples were below the EU recommended level of 50 µg/kg for the sum of T-2 and HT-2 in other cereals intended for direct human consumption [30]. In contrast, the concentration of T-2 in the *garri* samples (n=3) exceeded 15 µg/kg which is the EU recommended level for the sum of T-2 and HT-2 in cereal-based foods for infants and young children (EC, 2013). *Garri* is consumed by young children in Nigeria and in some cases, it serves as a weaning food especially among the low-income families which suggest that these children may be predisposed to the toxic effects of T-2.

DON and its acetylated (3-ADON and 15-ADON) and glucosylated (DON-3G) metabolites were detected in the samples. DON occurred in the food products (*garri*, *lafun*, and *amala*) at incidence rates of 38%, 28%, and 41% and maximum concentrations of 99 µg/kg, 91 µg/kg, and 125 µg/kg, respectively. 3-ADON and 15-ADON were not detected in the *garri* samples in contrast to *lafun* and *amala*. 15-ADON and FUS-X were detected in 8% of *lafun* samples while 12%, 6%, and 3% of *amala* samples were contaminated with 3-ADON, 15-ADON, and FUS-X, respectively. Of all the modified mycotoxins (DON and ZEN metabolites), only DON-3G was detected in the samples. DON-3G occurred in the *garri* samples at a range of 12 µg/kg to 20 µg/kg, and at an incidence rate of 13%. Although the incidence of modified mycotoxins has not been reported in tuber products from Nigeria, recent studies reported the occurrence of DON-3G in cereals and cereal products (*ogi*) from Nigeria [21,31].

The incidence rate and maximum levels of ZEN in *garri, lafun,* and *amala* were 17% (17 µg/kg), 6% (16 µg/kg), and 9% (19 µg/kg), respectively. Adejumo et al. [32] also reported ZEN as one of the major mycotoxins contaminating agricultural products in Nigeria. The occurrence of ZEN in other tuber crop products such as potato products has been reported [33]. In contrast, Somorin et al. [17] did not detect ZEN and DON in *amala* (yam flour) samples in their study. The variation in the results may be attributed to the sampling methods used. Somorin et al. [17] sampled whole yam chips while our samples consisted of finished *amala* products (yam flour). Microorganisms have a more easy accessibility to yam flour than yam chips because of the small particle size of yam flour as demonstrated by Ojokoh and Gabriel [34], who reported the presence of more spore forming units of fungi in yam flour during storage

compared to yam chips. Although the concentration of toxins in the different food types were below the EU limits for DON, FB, and ZEN in cereals intended for direct human consumption, there is still a safety concern because of the co-occurrence of multiple mycotoxins in the food products. Similarly, NEO, NIV, HT-2, α-ZEL, β-ZEL, and ZEN-14G were not detected in any of the food products.

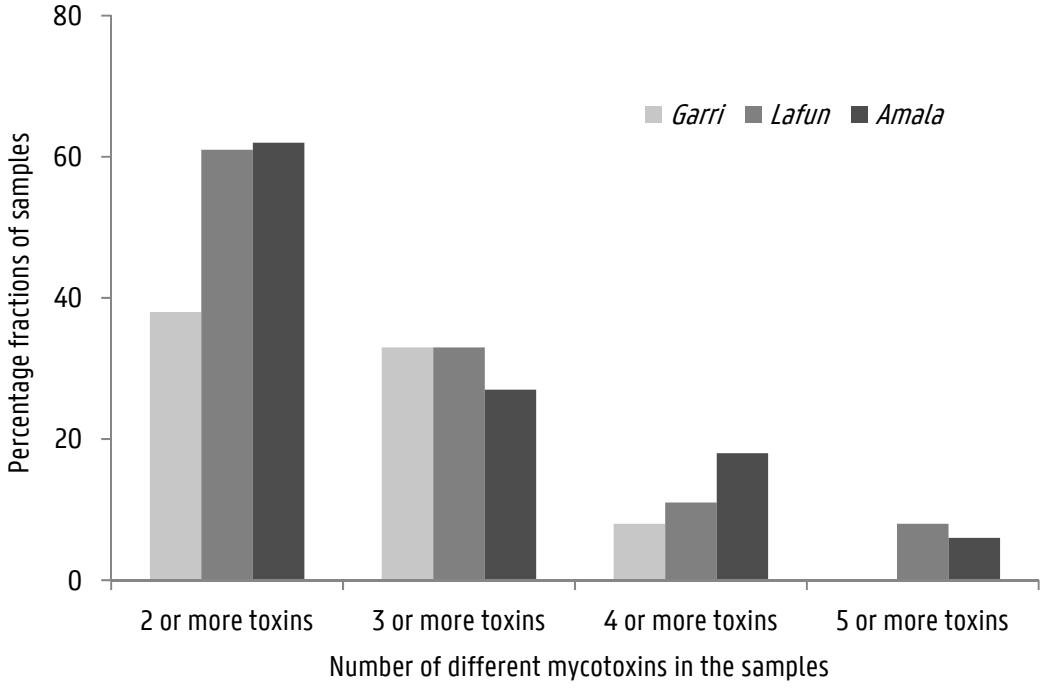

Figure 4.1 Distribution of number of *Fusarium* mycotoxins in cassava and yam products from Nigeria.

As observed in Figure 4.1, 62% of *amala* was contaminated with at least 2 mycotoxins out of the 17 mycotoxins investigated and this was followed by *lafun* (61%) and 38% of *garri*. A reasonable percentage of the different food products: *garri* (8%), *lafun* (11%), and *amala* (18%) were contaminated with at least 4 *Fusarium* mycotoxins. The frequency of mycotoxin co-occurrence in the samples suggests that a large proportion of the population, especially the young children are exposed to *Fusarium* mycotoxins through daily consumption of these food products.

4.4. Conclusion

The study reports the occurrence of *Fusarium* mycotoxins in some Nigerian staple foods: *garri*, *lafun*, and *amala*. Although the levels of the mycotoxins were not high, it is a concern because a large proportion of these products are consumed on daily basis by all the age groups in Nigeria. The situation is worsened by the co-occurrence of these toxins in the food products which may lead to synergistic health effects on the hosts. Effort should be channelled toward implementing good manufacturing and storage practices to prevent toxigenic fungal infestation and their associated mycotoxins. There is need for continuous monitoring and control of mycotoxins contamination in these products. To complete the Nigerian staple diets of interest in this PhD study, *Fusarium* mycotoxin contamination data of traditional spices and indigenous beans are described in Chapter 5.

4.5. References

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QUANTIFICATION OF *FUSARIUM* MYCOTOXINS IN NIGERIAN INDIGENOUS BEANS, SOYBEAN, AND THEIR PROCESSED PRODUCTS (TRADITIONAL SPICES AND SOYBEAN POWDER) USING MULTI-MYCOTOXIN LC-MS/MS METHOD

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CHAPTER 5: QUANTIFICATION OF *FUSARIUM* MYCOTOXINS IN NIGERIAN INDIGENOUS BEANS, SOYBEAN, AND THEIR PROCESSED PRODUCTS (TRADITIONAL SPICES AND SOYBEAN POWDER) USING MULTI-MYCOTOXIN LC-MS/MS METHOD

5.1 Introduction

SSA is endowed with rich culture, ethnic diversity, and agricultural resources thus leading to the production of a wide range of food and food products. Despite this diversity, SSA is the most food insecure part in the world [1]. Although there are several factors contributing to food insecurity in SSA, climate change seems a major driving factor because of the direct or indirect role it plays to the predominantly subsistence agricultural sector. The contamination of agricultural products by toxigenic fungi and mycotoxins is influenced by climate change [2]. Although there are no fixed figures ascribed to losses caused by mycotoxin contamination in SSA, especially Nigeria, the high incidence and concentration of mycotoxins in foods and food products from Nigeria (Chapters 3 and 4) correlates with significant losses of agricultural products. While there are several survey studies on the incidence of *Aspergillus* fungal metabolites especially aflatoxins in agricultural crops and products in Nigeria [3], data on the incidence of *Fusarium* mycotoxins and their modified forms remain seldom.

While a few number of reports on mycotoxin occurrence in agricultural crops such as cereal and cereal-based products in Nigeria exist [4–6], there are no reports on the incidence of mycotoxins in major traditional spices consumed in Nigeria. Traditional spices such as *dawadawa, ogiri,* and *okpehe* are produced by the fermentation of African indigenous beans including African locust bean (*Parkia biglobosa*), African castor bean (*Ricinus communis*), and African mesquite bean (*Prosopis africana*), respectively. They are mainly used as condiments in sauces, porridges, stews, and soups, and contribute significantly to the protein, vitamins, and energy intake of the large population of Nigerians [7]. Fermentation of *dawadawa*, *ogiri*, and *okpehe* is initiated by natural inoculation, thereby resulting to the contribution of a diverse group of organisms especially bacteria species such as *Bacillus*, *Enterobacteriaceae*, *Leuconostoc, Staphylococcus,* and *Micrococcus*. Other bacteria species that have been isolated during fermentation of traditional spices include *Escherichia, Pseudomonas,* and *Corynebacterium* [8]. Although toxigenic fungi do not contribute to the fermentation of these spices, studies have shown that these spices are often contaminated with toxigenic fungi [9,10] because of the fraudulent used of poor quality raw materials. Another possible route of contamination of these products by toxigenic fungi is the unhygienic and uncontrolled fermentation conditions, poor storage conditions, and high moisture content of these products, thus favouring the

growth of toxigenic organisms. A recent study by Adekoya et al. [11] also isolated *Fusarium, Aspergillus,* and *Penicillium* from Nigerian traditional spices.

In addition to the indigenous beans, soybean (*Glycine max*) also plays a vital role in Nigeria. It may be processed into soybean powder, milk, oil, and animal feeds. Soybean powder is the most common food product that serves as a complementary weaning food in Nigeria. It is produced either by roasting the beans and then milling into powder or by blanching, sun-drying and milling of the beans. Soybean is known for its high protein content and presents a healthy formulation compliment of infant meals towards combating malnutrition. The combination of soybean powder and cereal-based product - *ogi* (described in Chapter 3) serves as the first weaning food for infants, both the rural and urban population. Notwithstanding the importance of soybean and soy products, data on occurrence of *Fusarium* mycotoxins are limited. Only a few studies have been conducted on the incidence of fungi and mycotoxins in soybean and its products especially in Nigeria despite its high consumption as an infant complementary food [12–14].

Due to the paucity of information on mycotoxin occurrence in traditional spices and soybean products, as well as the high consumption rate of these products in Nigeria, there is a need to examine the safety of these products in respect to contamination with fungal metabolites. Thus, the present study investigated the occurrence of *Fusarium* mycotoxins in traditional spices (*dawadawa, ogiri*, and *okpehe*) and processed soybean powder from Nigerian markets. To further get insights on the occurrence of *Fusarium* mycotoxins beyond the focus products, the raw materials -soybean and the indigenous beans (African locust beans (ALB), African castor beans (ACB), and African mesquite beans (AMB)) used in the production of soybean powder and spices were also investigated for the occurrence of *Fusarium* mycotoxins.

5.2 Materials and methods

5.2.1. Sample collection

In total 192 samples including indigenous beans (ALB, n=30, AMB, n=21, and ACB, n=21), traditional spices (*dawadawa*, n=17, *okpehe*, n=21, and *ogiri*, n=20), soybean (n=30) and processed soybean powder (n=32) were collected from various markets in DS and HF zones of Nigeria between September 2015 and October 2015. The sampling states were determined based on the major producing areas of the crops and food products (Table 5.1). Samples were thoroughly

homogenised, and representative portions of 200 g of dry samples were taken, packed in a transparent zip lock bag and transported to the Laboratory of Food Analysis (Ghent University, Belgium) for further analysis. Samples that were not immediately used, were stored at -20 °C until required.

Table 5.1 Sampling sites of indigenous beans, soybean, and their processed products from Nigerian markets

Group	Product Type	AEZ	State	No. of Towns	No. of Markets	No. of Samples
Indigenous beans	African locust bean	HF	Anambra	4	8	30
	African castor bean	HF	Anambra	4	8	21
	African mesquite bean	DS	Benue	3	6	21
Traditional spices	*Dawadawa*	HF	Anambra	4	8	17
	Ogiri	HF	Anambra	4	8	20
	Okpehe	DS	Benue	3	6	21
Soybean and product	Soybean	DS	Benue	3	6	30
	Processed soybean powder	DS	Benue	3	6	32
Total Number of Samples						192

AEZ = agro-ecological zone, HF = Humid Savanna, DS = Derived Savanna

5.2.2. Chemicals and Reagents

All the chemicals and reagents used for sample preparation and LC-MS/MS analysis were of analytical grade, and their sources and preparations are the same as described in Chapter 3 (Section 3.2.2).

5.2.3. Mycotoxin Analysis

Sample preparation and extraction of samples were done as described in Chapter 3 (Section 3.2.3). The instrumentation and the conditions used for detection and quantification of *Fusarium* mycotoxins in both the indigenous beans, traditional spices, soybean and processed soybean powder have been reported by Monbaliu et al. [15] and are the same as described in Chapter 3 (Section 3.2.6). The data on the method performance characteristics including the limit of detection and limit of quantification for the detected compounds in the different food matrices are presented in Table 5.2. The apparent recovery for each mycotoxin was determined by dividing the observed value by spiked levels. The obtained values were between 70% and 102% which is in conformity with the ranges set in legislation [16].

Table 5.2. Limits of detection (LOD) and limits quantification (LOQ) of the *Fusarium* mycotoxins as obtained in the LC-MS/MS analysis of indigenous beans, traditional spices, and soybean.

	LOD (µg/kg)							LOQ (µg/kg)						
Mycotoxin	ALB	AMB	ACB	*Dawadawa*	*Okpehe*	*Ogiri*	soybean	ALB	AMB	ACB	*Dawadawa*	*Okpehe*	*Ogiri*	soybean
FB_1	13.0	12.1	14.5	12.0	14.9	12.2	13.1	26.0	24.2	29.0	24.0	29.8	24.4	26.2
FB_2	17.7	11 .2	14.0	13.8	10 .3	14.1	11.2	35.4	22.4	28.0	27.6	20.6	28.2	22.4
FB_3	15.9	23.0	19.0	20.0	20.0	20.4	16.6	31.8	46.0	38.0	40.0	40.0	40.8	33.2
DON	18.0	13.8	9.6	14.0	14.4	18.1	13.3	36.0	27.6	19.2	28.0	28.8	36.2	26.6
3-ADON	5.3	13.1	11.7	12.4	11.5	11.6	3.6	10.6	26.2	23.4	24.8	23.0	23.2	7.2
15-ADON	7.1	7.0	8.0	6.0	8.4	6.1	2.5	14.2	14.0	16.0	12.0	16.8	12.2	5.0
DON-3G	15.7	13.4	13.0	14.3	14 .3	14.1	2.7	31.4	26.8	26.0	28.6	28.6	28.2	5.4
ZEN	4.4	6.2	6.0	4.0	10.1	6.0	3.6	8.8	12.4	12.0	8.0	20.2	12.0	7.2
α- ZEL	5.1	6.2	6.3	5.4	5.3	5.2	5.0	10.2	12.4	12.6	10.8	10.6	10.4	10.0
β-ZEL	8.3	8.6	8.4	6.4	7.0	7.3	5.4	16.6	17.2	16.8	12.8	14.0	14.6	10.8
ZEN-14G	9.0	9.0	8.9	7.1	7.3	7.0	5.8	18.0	18.0	17.8	14.2	14.6	14.0	11.6
T-2	8.0	10.3	10.3	11.1	8.6	9.4	13.4	16.0	20.6	20.6	22.2	17.2	18.8	26.8
HT-2	8.3	7.0	6.3	5.3	5.5	5.0	4.4	16.6	14.0	12.6	10.6	11.0	10.0	8.8
FUS-X	63.6	64.0	54.0	58.0	70.2	62.1	32.9	127.2	128.0	108.0	116.0	140.4	124.2	65.8
NIV	85.2	80.8	86.0	82.2	73.0	76.4	48.1	170.4	161.6	172.0	164.4	146.0	152.8	96.2
DAS	1.8	1.6	2.0	1.4	2.0	1.4	0.87	3.6	3.2	4.0	2.8	4.0	2.8	1.7
NEO	4.0	5.0	4.3	3.9	3.9	5.2	3.5	8.0	10.0	8.6	7.8	7.8	10.4	7.0

LOD = Limit of detection, LOQ = Limit of quantification, ALB = African locust beans, ACB = African castor beans, AMB = African mesquite beans, FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, T-2 = T-2 toxin, HT-2 = HT-2 toxin, FUS-X = fusarenon-X, NIV = nivalenol, DAS = diacetoxyscirpenol, NEO = neosolaniol.

The LOD, LOQ, and apparent recovery of soybean were employed for the processed soybean powder.

5.2.4. Data Analysis

Descriptive analysis of the data was performed using Microsoft office Excel 2007 (Redmond, WA, USA) and SPSS statistical package version 23 (SPSS Inc., Chicago, Illinois, USA).

5.3 Results and discussion

5.3.1. Occurrence of *Fusarium* mycotoxins in soybean and processed soybean powder

Although soybean and its products are seen as low risk products for toxigenic fungi and mycotoxins, the current study reports a wide range of *Fusarium* mycotoxins in soybean and soybean products from the Nigerian markets. A similar study showing high susceptibility of soybean to mycotoxins was reported in Cameroon [17]. In general, processed soybean powder (PSP) samples were observed to be more contaminated with *Fusarium* mycotoxins as compared to the soybean samples, with 12 *Fusarium* mycotoxins being detected in PSP while the soybean samples were contaminated with 7 *Fusarium* mycotoxins (Figure 5.1, Table 5.3). Overall, 47% and 100% of soybean and PSP, respectively, were contaminated with at least one mycotoxin. This result is unexpected based on the reported scientific facts that mycotoxin contamination is reduced during food processing. However, fungal growth and mycotoxin contamination may continue in processed food products when products are exposed to poor storage conditions which are favourable for fungi to strive. Furthermore, improper packaging of PSP may lead to absorption of water from the environment because of their high surface area, thus increasing the moisture content and risk of fungal infestation. High incidence of mycotoxins in PSP samples compared to soybean samples may also be attributed to the processing of these products under poor and unhygienic environment as well as the fraudulent use of poor quality grade of soybean seed for the processing of PSP (see Chapter 7 for more details on PSP processing).

ZEN was the predominant mycotoxin in both food types occurring at an incidence rate of 30% (range: 8-30 µg/kg) and 81% (27-388 µg/kg) in soybean and PSP samples, respectively (Table 5.3). Earlier study on the propensity of *Fusarium* species isolated from Nigerian soybean to produce ZEN reported *F. equiseti* as the highest producer of ZEN [14] suggesting that our samples may have been contaminated with *F. equiseti.* ZEN is classified as a non-steroidal estrogenic mycotoxin because of its link with reproductive problems in several animal spp. and probably humans (Chapter 1). Fortunately, levels of ZEN detected in soybean samples were below the EU maximum limit for ZEN (100 µg/kg) in cereals intended for direct human consumption [16]. On the other hand, a reverse trend was observed for

PSP samples with a maximum concentration of 388 µg/kg. Processed soybean powder is used as major source of protein in foods for the infants and young children in Nigeria. Although there is no maximum limit set for ZEN in SSA, the EU maximum limit for ZEN in foods for infants and children is 20 µg/kg. Approximately 52% of the total samples analysed in this study exceeded 20 µg/kg, with PSP samples contributing 81% of the quote. The high incidence of ZEN observed especially in PSP is comparable to the rate (100%) reported in soybean by Abia et al. [17]. Similar observations on the occurrence of ZEN in soybean and soybean products have previously been reported [18–20] which suggest possible contamination of soybean with pathogenic fungi.

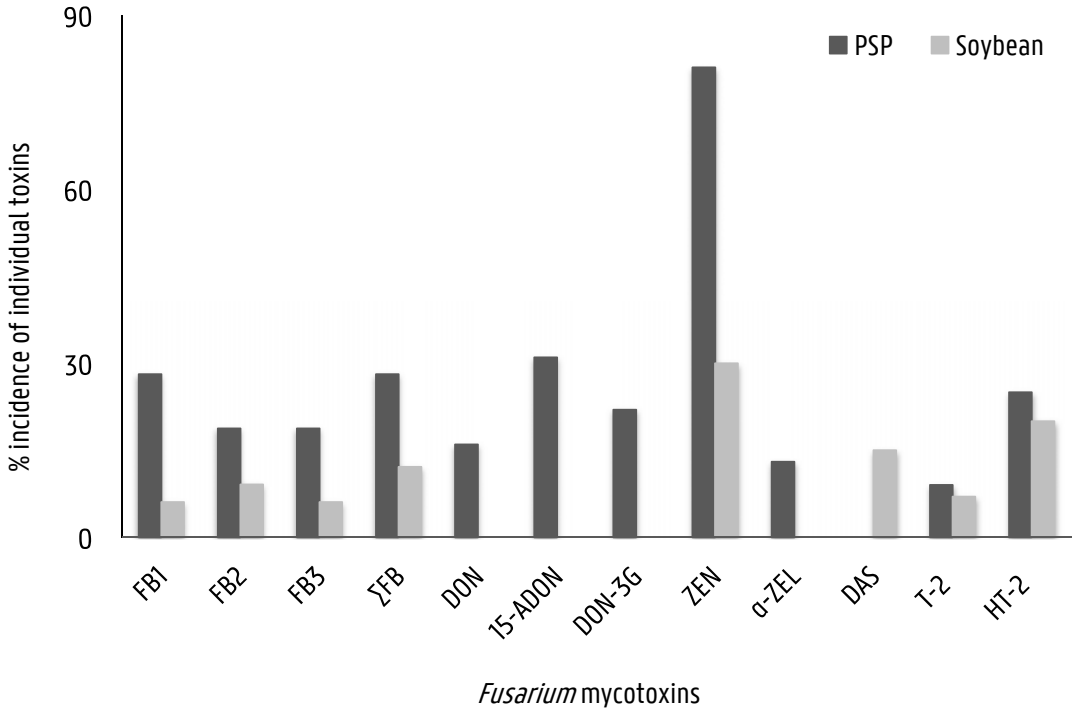

Figure 5.1 Incidence of *Fusarium* mycotoxins in soybean and processed soybean powder (PSP) samples from Nigeria. FB_1, B_2, B_3 = fumonisin B_1, B_2, B_3; ∑FB = fumonisin $B_1 + B_2 + B_3$; DON = deoxynivalenol; 15-ADON = 15-acetyl-DON; DON-3G = DON-3-glucoside; ZEN = zearalenone; α-ZEL = α-zearalenol; DAS = diacetoxyscirpenol; T-2 = T-2 toxin; and HT-2 = HT-2 toxin

Table 5.3 Percentage of positive samples, concentration range, mean concentration, and median concentration (µg/kg) of *Fusarium* mycotoxins in soybean and processed soybean powder from the Nigerian markets

	Soybean (n=30) (µg/kg)				Processed soybean powder (n=32) (µg/kg)			
Mycotoxin	No +ve (%+ve)	Range	Mean ± SD	Med	No +ve (%+ve)	Range	Mean ± SD	Med
FB_1	2 (7)	52 - 53	53 ± 0.68	53	9 (28)	34 - 270	116 ± 65	99
FB_2	3 (10)	48 - 67	57 ± 9.1	57	6 (19)	44 - 181	89 ± 51	71
FB_3	2 (7)	39 - 40	40 ± 0.56	40	6 (19)	39 - 56	46 ± 5.7	44
∑FBs	4 (13)	53 - 140	89 ± 42	82	9 (28)	34 - 500	206 ± 137	187
DON	0	na	na	na	5 (16)	61 - 180	102 ± 48	85
15-ADON	0	na	na	na	10 (31)	71 - 113	95 ± 13	96
DON-3G	0	na	na	na	7 (22)	12 - 14	13 ± 0.7	13
ZEN	9 (30)	8 - 30	21 ± 6.9	22	26 (81)	27 - 388	60 ± 70	38
α-ZEL	0	na	na	na	4 (13)	19 - 22	21 ± 1.3	21
DAS	5 (17)	2 - 5	4 ± 1.2	3.5	0	na	na	na
T-2	2 (7)	21 - 23	22 ± 1.3	22	3 (9)	10 - 13	11 ± 1.6	11
HT-2	6 (20)	16 - 25	21 ± 3.1	20	8 (25)	22 - 35	29 ± 4.7	29
FUS-X	0	na	na	na	7 (22)	66 - 276	124 ± 73	93
NIV	0	na	na	na	7 (22)	70 - 104	83 ± 12	79

No+ve = number of positive samples, %+ve = percentage positive samples, Mean = mean concentration; SD = standard deviation; Med = median concentration; FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; ∑FBs = fumonisin B_1 + B_2 + B_3; DON = deoxynivalenol; 15-ADON = 15-acetyl-deoxynivalenol; DON-3G = deoxynivalenol-3-glucoside; ZEN = zearalenone; α-ZEL = α-zearalenol; DAS = diacetoxyscirpenol; T-2 = T-2 toxin; HT-2 = HT-2 toxin; FUS-X = fusarenone-X; NIV = nivalenol; na = not applicable. Mean, median, and concentration range were calculated based on positive samples only.

This is evident in the studies of Levic et al. [21] and Medić-Pap et al. [22] which revealed the incidence of *Fusarium* fungal species in soybean and soybean products. In addition, Ezekiel et al. [14] reported the high potential of *Fusarium* fungi (*F. equiseti*) isolated from Nigerian soybean in producing ZEN. Evidence of the occurrence of ZEN and its metabolites in other Nigerian foodstuffs has also been reported [6,23]. Of the ZEN metabolites analysed, none of them were detected in soybean samples, whereas only α-ZEL was detected in PSP samples at an incidence rate and mean concentration of 13% and 21 µg/kg, respectively. This fact corroborates with the study of Abia et al. [17].

Other EU regulated mycotoxins detected in the samples were the FBs. The incidence of FBs was more dominant in the PSP samples compared to soybean samples. FB_1, FB_2, and FB_3 occurred in 28%, 19%, and 19% of PSP samples, respectively, whereas in soybean, the percentage incidence was 7% (FB_1), 10% (FB_2), and 7% (FB_3). The concentration and incidence rate of FB_2 in soybean samples in the present study were higher compared to FB_1 and FB_3 (Table 5.3) which suggests the possible contamination of the analysed samples with *A. niger*. Recent studies on food commodities from Nigeria have revealed this trend, and as such attention should be given to elucidate the morphology and potential of *A. niger* producing FB_2 in this region. The pattern of FBs contamination in PSP samples was in contrast with soybean samples with FB_1 being the dominant one. Occurrence of FBs in soybean and soybean products have previously been reported by several authors [13,17,18,24,25] although the rate observed in the present study is lower compared to that reported in the previous studies. Njobeh et al. [16] reported a 40% incidence rate of FB_1 (range: 25 - 365 µg/kg, mean: 196 µg/kg) in soybean samples from Cameroon. While the levels of FBs in this study were below the EU maximum limit of 1000 µg/kg set for maize intended for direct human consumption, it is worrisome that 4 samples of PSP exceeded the EU level for FBs in foods for infants and children.

Both type A and type B trichothecenes (TH) were also detected in the samples. Surprisingly, no type B TH was detected in the soybean samples in contrast to the results of the PSP samples which were contaminated with DON, 15-ADON, DON-3G, FUS-X, and NIV at an incidence rate and mean concentration of 16% (102 µg/kg), 31% (95 µg/kg), 22% (3 µg/kg), 22% (124 µg/kg), and 22% (83 µg/kg), respectively. Interestingly, DON metabolites (15-ADON and DON-3G) occurred at a higher rate in PSP as compared to DON. The incidence rate of DON as observed was lower as compared to the previous study by Gutleb et al. [24], who recorded 72.2% incidence of DON in soy feed samples although at a low concentration. Although there are no studies from SSA on the occurrence of a wide range of *Fusarium* mycotoxins in processed soybean powder, Abia et al. [17] also reported a 100% incidence rate of DON (56 – 75 µg/kg), DON-3G (<LOQ – 1 µg/kg), and FUS-X (range: 33 – 42 µg/kg) in soybean samples from Cameroon. The authors also detected NIV in 90% of their samples. Among the type A TH, T-2 and HT-2 were detected in the two

sample types at an incidence rate of 7% (range: 21 - 23 µg/kg) and 20% (range: 16 - 25 µg/kg) for soybean samples, and 9% (10 - 13 µg/kg) and 25% (22 - 35 µg/kg) for PSP samples, respectively. Based on the EU recommendation level (15 µg/kg) for the sum of T-2 and HT-2 in cereal-based foods for infants and young children, this group of the population in Nigeria may be exposed to the toxigenic health effects of T-2 and HT-2 since 20% and 25% of soybean samples and processed soybean samples exceeded the limit, respectively.

Irrespective of the sample type, an appreciable percentage of the PSP samples (81%) and soybean samples (33%) were contaminated with at least two mycotoxins (Table 5.4). The co-occurrence of toxins in PSP sample was higher compared to soybean samples. Approximately 50% of PSP had up to 3 mycotoxins as against 13% of soybean samples. Co-occurrence of mycotoxins in PSP was higher between ZEN and 15-ADON at 28%, succeeded by ZEN and FB_1 (22%). DON, DON-3G, and FUS-X co-occurred with ZEN at the rate of 9%, 16%, and 19%, respectively. Three samples of PSP were contaminated with up to 7 mycotoxins. Abia et al. [26] reported a complex mixture of mycotoxins occurring in soybean samples with about 21 fungal metabolites contaminating one sample. This study reveals for the first time the co-occurrence of *Fusarium* mycotoxins in PSP and actions are needed to checkmate the raw material used in processing PSP, storage conditions under which this product is stored after processing, as well as the packaging material to control mycotoxins in this product, and protect children from toxic effects of these toxins.

Table 5.4 Co-occurrence of *Fusarium* mycotoxins in soybean and processed soybean powder samples from Nigeria

No of toxins per sample	Soybean (n=30)		Processed soybean powder (n=32)	
	No of samples	Percentage (%)	No of samples	Percentage (%)
2	6	20	11	34
3	3	10	4	13
4	1	3	4	13
5	na	na	3	9
6	na	na	1	3
7	na	na	3	9

na = not applicable

5.3.2 Occurrence of *Fusarium* mycotoxins in traditional spices and indigenous beans

This study reports for the first time the incidence of a wide range of mycotoxins in traditional spices and their indigenous beans including ALB, ACB, and AMB (Tables 5.5 and 5.6). In total, 58 samples of traditional spices and 72

samples of indigenous beans were analysed for different *Fusarium* metabolites. The present study revealed that these food commodities were contaminated with multiple *Fusarium* mycotoxins, and up to 10 and 13 *Fusarium* metabolites were detected in the traditional spices and the indigenous beans, respectively. FBs, ZEN, and DAS were the most prevalent mycotoxins occurring in all the sample types.

FBs were detected in samples of ALB, ACB, and AMB at a prevalence rate of 70%, 62%, and 52%, respectively and a mean value of 372 µg/kg (range: 103 µg/kg - 1010 µg/kg), 236 µg/kg (range: 68 µg/kg - 377 µg/kg), and 155 µg/kg (range: 85 µg/kg - 358 µg/kg) (Table 5.5). Of the FBs, FB_2 was the most dominant occurring in 70%, 62%, and 38% of ALB, ACB, and AMB, respectively, which is in contrast with the natural occurrence ratio of FBs (FB_1:FB_2:FB_3, 10:3:1) reported by the Joint FAO/WHO Expert Committee on Food Additives [27]. FB_1 was also detected in 63%, 48%, and 29% at a range of 79 µg/kg to 344 µg/kg, 87 µg/kg to 211 µg/kg, and 98 µg/kg to 192 µg/kg, respectively. Samples of AMB were negative for FB_3, while a range of 49 µg/kg to 79 µg/kg and 58 µg/kg to 80 µg/kg of FB_3 were detected in 27% and 29% of ALB and ACB, respectively. The same trend was observed in the traditional spices with the sum of FBs (FB_1+FB_2+FB_3) ranging from 49 µg/kg to 287 µg/kg, except for *ogiri* samples which were contaminated with only FB_2 at a range of 96 µg/kg to 151 µg/kg, and an incident rate of 20% (Table 5.6). The prevalence of FBs in food commodities from Nigeria has previously been reported (Chapter 3). Although FB_1 is the most dominant FBs occurring in nature, recent studies have revealed the possible occurrence of only FB_2 in food samples as observed in the present study and corroborates the reports of Chapter 3, Chapter 4, and Ezekiel et al. [28] on the occurrence of only FB_2 in some cereal samples. The occurrence of only FB_2 can be attributed to the contamination of the food commodities with *A. niger* which is a principle producer of FB_2 [29,30]. Several studies have reported *A. niger* as one of the major fungal species contaminating ALB, ACB and traditional spices (*dawadawa, okpehe*, and *ogiri*) [9,10,31–34], however, it was not confirmed in the present study.

As regard to regulated mycotoxins, ZEN was detected in all the sample types at a range of 21 µg/kg to 194 µg/kg for the indigenous beans and 33 µg/kg to 115 µg/kg for the traditional spices. The incidence rate of ZEN in the indigenous beans (ALB-70%, ACB-43%, and AMB-67%) was higher compared to that of the traditional spices (*dawadawa*-35%, *ogiri*-20%, and *okpehe*-19%). Although there are no previous reports on the occurrence of ZEN in traditional spices and the indigenous beans in Nigeria, the incidence of *Fusarium* fungal species have been reported in these products [9,32–34]. In addition, α-ZEL was also detected in ACB at a lower incidence rate of 14% and a concentration range of 20 µg/kg to 23 µg/kg with a mean concentration of 21 µg/kg.

Table 5.5 Percentage of positive samples, concentration range, mean concentration, and median concentration (µg/kg) of *Fusarium* mycotoxins in indigenous bean samples from Nigeria

	African locust bean (n=30)				African castor bean (n=21)				African mesquite bean (n=21)			
Mycotoxin	No +ve (%+ve)	Range	Mean ± SD	Med	No +ve (%+ve)	Range	Mean ± SD	Med	No +ve (%+ve)	Range	Mean ± SD	Med
FB_1	19 (63)	79 - 344	131 ± 65	111	10 (48)	87-211	137 ± 41	127	6 (29)	98-192	147 ± 37	154
FB_2	21 (70)	54 -841	229 ± 241	141	13 (62)	66-173	98 ± 34	89	8 (38)	85-166	104 ± 27	94
FB_3	8 (27)	49 - 79	64 ± 13	68	6 (29)	58-80	71 ± 9.3	72	0	na	na	na
∑FBs	21 (70)	103-1010	372 ± 255	253	13 (62)	68-377	236 ± 95	253	11 (52)	85-358	155 ± 87	115
DON	2 (7)	94 - 98	96 ± 2.6	96	3 (14)	108-228	165 ± 60	158	5 (24)	82-288	190 ± 76	206
15-ADON	4 (13)	10 - 13	48 ± 1.4	12	0	na	na	na	7 (33)	15-58	35 ± 17	29
DON-3G	0	na	na	na	0	na	na	na	10 (48)	6-36	14 ± 9.2	10
ZEN	21 (70)	54 - 194	118 ± 45	96	9 (43)	57-95	76 ± 15	85	14 (67)	21-116	62 ± 26	58
α-ZEL	0	na	na	na	3 (14)	20-23	21 ± 2.1	20	0	na	na	na
DAS	10 (33)	10 - 33	15 ± 6.8	13	2 (10)	5-6	6 ± 0.8	5.7	14 (67)	2-9	6 ± 2.1	7
T-2	2 (7)	10 - 11	10 ± 0.3	10	0	na	na	na	0	na	na	na
HT-2	0	na	na	na	7 (33)	39-82	57 ± 15	52	7 (33)	88-121	99 ± 12	98
FUS-X	6 (20)	76 - 151	105 ± 28	99	0	na	na	na	8 (38)	98-142	120 ± 15	118
NIV	11 (37)	68 - 306	123 ± 69	92	2 (10)	95-106	100 ± 7.8	100	0	na	na	na

No+ve = number of positive samples; % + ve = percentage positive samples; mean = mean concentration; SD = standard deviation; Med = median concentration; FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; ∑FB = fumonisin B_1 + B_2 + B_3; DON = deoxynivalenol; 15-ADON = 15-acetyl-deoxynivalenol; DON-3G = deoxynivalenol-3-glucoside; ZEN = zearalenone; α-ZEL = α-zearalenol; DAS = diacetoxyscirpenol; T-2 = T-2 toxin; HT-2 = HT-2 toxin; FUS-X = fusarenone-X; NIV = nivalenol; na = not applicable. Mean, median, and concentration range were calculated based on positive samples only.

Table 5.6 Percentage of positive samples, concentration range, mean concentration, and median concentrations (µg/kg) of *Fusarium* mycotoxins in traditional spices from Nigeria

	Dawadawa (n=17)				*Ogiri* (n=20)				*Okpehe* (n=21)			
Mycotoxin	No +ve (%+ve)	Range	Mean ± SD	Med	No +ve (%+ve)	Range	Mean ± SD	Med	No +ve (%+ve)	Range	Mean ± SD	Med
FB_1	8 (47)	72-165	100 ± 28	90	0	na	na	na	7 (33)	94-182	131 ± 34	127
FB_2	10 (59)	43-170	87 ± 52	57	4 (20)	96-151	129 ± 24	134	5 (24)	79-142	103 ± 25	96
FB_3	3 (18)	26-131	87 ± 48	93	0	na	na	na	2 (10)	51-77	64 ± 18	64
∑FBs	13 (77)	49-287	148 ± 63	156	4 (20)	96-151	129 ± 24	134	7 (33)	151-274	224 ± 50	227
DON	0	na	na	na	3 (15)	90-155	117 ± 34	106	7 (33)	90-164	125 ± 28	118
15-ADON	4 (24)	16-29	21 ± 6	20	0	na	na	na	0	na	na	na
ZEN	6 (35)	33-86	55 ± 23	49	4 (20)	53-83	71 ± 13	74	4 (19)	78-115	98 ± 19	100
DAS	3 (18)	3-6	5 ± 1.5	5	2 (10)	5-6	5 ± 0.9	5	5 (24)	10-32	20 ± 9.9	16
T-2	6 (35)	21-32	27 ± 4.1	27	0	na	na	na	1 (5)	52	52	52
HT-2	1 (6)	58	58	58	0	na	na	na	0	na	na	na
NIV	0	na	na	na	3 (15)	126-358	256 ± 118	285	0	na	na	na

No+ve = number of positive samples; % + ve = percentage positive samples; mean = mean concentration; SD = standard deviation; Med = median concentration; FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; ∑FBs = fumonisin B_1 + B_2 + B_3; DON = deoxynivalenol; 15-ADON = 15-acetyl-deoxynivalenol; ZEN = zearalenone; DAS = diacetoxyscirpenol; T-2 = T-2 toxin; HT-2 = HT-2 toxin; NIV = nivalenol; na = not applicable. Mean, median, and concentration range were calculated based on positive samples only.

Overall, 14% of the indigenous beans were contaminated with DON at percentage incidences of 7% (max-98 µg/kg), 14% (max-228 µg/kg), and 24% (max-288 µg/kg) for ALB, ACB, and AMB, respectively (Table 5.5). DON was also detected in 3 and 7 samples of *ogiri* and *okpehe* with a mean value of 117 µg/kg (max-155 µg/kg) and 125 µg/kg (max-164 µg/kg), respectively (Table 5.6). The acetylated form of DON (15-ADON) was detected in ALB, AMB, as well as in *dawadawa* at a range of 10 µg/kg -13 µg/kg (13%), 15 µg/kg -58 µg/kg (33%), and 16 µg/kg -29 µg/kg (24%). In addition, DON-3G was detected in approximately half of AMB samples at a mean concentration of 14 µg/kg (range: 6-36 µg/kg). Other type B TH present in the samples were FUS-X and NIV. FUS-X occurred in the indigenous beans (ALB and AMB) at a mean concentration of 105 µg/kg (20%) and 120 µg/kg (38%), respectively. Similarly, NIV occurred in ALB, ACB, and *ogiri* at ranges between 68 µg/kg and 306 µg/kg, 95 µg/kg and 106 µg/kg, and 126 µg/kg and 358 µg/kg, respectively. Although Nigeria has paid little attention to the occurrence of TH in food commodities, there is evidence of occurrence of these toxins in Nigerian food commodities (Chapter 3 and 4). Type A TH (T-2, HT-2, and DAS) were also detected in the different sample types. Interestingly, DAS was found to occur in all the sample types at an overall percentage incidence rate of 36% (2 µg/kg - 33 µg/kg) and 17% (3 µg/kg - 32 µg/kg) for the indigenous beans and traditional spices, respectively. Adejumo et al. [35] also reported 9.4% incidence rate of DAS in food commodities from Nigeria which corroborates with the trend observed in Nigerian cereals (Chapter 3). However, there is need for more studies to identify the toxigenic fungi responsible for DAS production in Nigeria. T-2 occurred only in 7% of ALB and in 35% and 5% of *dawadawa* and *okpehe*, respectively. A series of human and animal diseases, ranging from acute to chronic have been associated to consumption of TH contaminated food and feed (Chapter 1) which makes its occurrence in Nigerian spices a possible health problem.

About 78% and 47% of the indigenous beans and traditional spices, respectively, were contaminated with at least 2 toxins, with AMB (95%) and *dawadawa* (65%) having the highest percentage (Table 5.7). Co-occurrence of *Fusarium* mycotoxins was higher in the indigenous beans with up to 8 and 7 toxins contaminating ALB and AMB, respectively. ACB was less contaminated as compared to ALB and AMB, with the highest prevalence of 5 toxins in 4 samples. A similar trend was observed in respect to the spices with *ogiri* (a product from ACB) having the least co-occurrence rate.

Table 5.7 Co-occurrence of *Fusarium* mycotoxins in traditional spices and indigenous beans samples from Nigeria

No of toxins per sample	ALB (n=30)		ACB (n=21)		AMB (n=21)		*Dawadawa* (n=17)		*Ogiri* (n=20)		*Okpehe* (n=21)	
	No of samples	Percentage (%)	No of samples	Percentage (%)	No of samples	Percentage (%)	No of samples	Percentage (%)	No of samples	Percentage (%)	No of samples	Percentage (%)
2	0	0	3	14	3	14	2	12	5	25	3	14
3	3	10	3	14	5	24	5	29	2	10	5	24
4	6	20	5	24	5	24	2	12	0	0	1	5
5	6	20	4	19	4	19	2	12	0	0	0	0
6	2	7	0	0	2	10	0	0	0	0	0	0
7	3	10	0	0	1	5	0	0	0	0	0	0
8	1	3	0	0	0	0	0	0	0	0	0	0

Indigenous beans = African locust bean (ALB), African castor bean (ACB), African mesquite bean (AMB), traditional spices = *dawadawa, ogiri*, and *okpehe.*

5.4 Conclusion

The present study gives a detailed profile of *Fusarium* mycotoxins contaminating popular traditional spices (*dawadawa, ogiri*, and *okpehe*) and processed soybean powder consumed in Nigeria. In addition, a summary of *Fusarium* mycotoxins present in three indigenous beans (ALB, ACB, and AMB) used in processing of *dawadawa, ogiri*, and *okpehe* was reported for the first time. The study revealed high incidence (overall - 77%) of *Fusarium* mycotoxins in these food products. The observed trend in contamination followed the pattern reported in Nigerian food commodities such as in cereal and cereal-based products (Chapter 3) and tuber products (Chapter 4). Available information shows that no studies have investigated the occurrence of a wide range of *Fusarium* mycotoxins and their modified forms on different food commodities in Nigeria. Co-occurrence of mycotoxins is of concern because of the increased risk of synergistic effects on the host as reviewed in Chapter 1. This necessitates the need to constantly monitor as well as adopt mitigation strategies towards the control of *Fusarium* mycotoxins in Nigerian food crops and products. In addition, further studies are needed to ascertain the possible route of contamination of food products by *Fusarium* mycotoxins. The behaviour of *Fusarium* mycotoxins along the process chain of the traditional spices (*dawadawa, ogiri*, and *okpehe*) and processed soybean powder is described in Chapters 6 and 7.

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STABILITY OF FUMONISIN B_1, DEOXYNIVALENOL, ZEARALENONE, AND T-2 TOXIN DURING PROCESSING OF TRADITIONAL BEERS AND SPICES

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CHAPTER 6: STABILITY OF FUMONISIN B_1, DEOXYNIVALENOL, ZEARALENONE, AND T-2 TOXIN DURING PROCESSING OF TRADITIONAL BEERS AND SPICES

6.1. Introduction

Mycotoxins are chemically stable compounds, however, studies have shown that food processing can influence their levels in food products (Chapter 1, Section 1.5). The impact on the mycotoxin concentration is dependent on several factors such as the type of processing, the mycotoxin, as well as the initial concentration of the contaminant in the food matrix. Although, there are many studies on the influence of processing on *Fusarium* mycotoxins, there are still disparity in results as to the effect of processing on mycotoxins. In the case of DON, studies have reported its degradation as a result of fermentation during bread making [1], however, Valle-Algarra et al. [2] and Bergamini et al. [3] observed a stability and even increase, respectively. Lancova et al. [4] reported a decrease in beer as compared to malting during brewing while an earlier study by Niessen and Donhauser reported an opposite trend [5]. In addition, the issue of modified mycotoxins has contributed to the complexity of this subject. During processing the structure of free mycotoxins may be altered, thus releasing an amalgam of structurall- related compounds [6]. An increase in the DON-3G as compared to DON was observed during beer production [4]. On the other hand, ZEN was metabolised to α-ZEL and β-ZEL during brewing as a result of the activity of *Saccharomyces cerevisiae* [4,7]. This trend was also observed by Keller et al. [8], who investigated the *in vitro* performance of *S. cerevisiae* strains isolated from bovine forages for the detoxification of ZEN, α-ZEL, and β-ZEL. Although the metabolisation of free mycotoxins to modified mycotoxins has been reported as a possible detoxification method, modified mycotoxins may be hydrolysed in the gastrointestinal tract [9], thus, representing a potential additional risk for both animals and humans.

Fermentation is one of the most convenient processing methods utilised both in rural and urban areas of Nigeria to primarily prolong the shelf-life of food as well as to create new food varieties. However, the problem is the rudimentary and uncontrolled processing environment which may encourage further growth of numerous microbes and *Fusarium* fungi and consequent production of mycotoxins. Previous Chapters (Chapters 3 and 5) revealed the contamination of Nigerian fermented food products such as traditional beers and traditional spices as well as their raw materials with multiple *Fusarium* mycotoxins. Hence exposure to mycotoxins by consumers of these products should not be underestimated. So far, only limited studies have elucidated the effect of processing on the stability of mycotoxins in Nigerian fermented food products. Specifically, Ezekiel et al. [10] investigated the effect of processing on Nigerian fermented beverages considering the raw cereals, their malted forms, and the final drinks. As a follow-up, there is a need to study the effects of the detailed processing steps

of traditional beverages on mycotoxins especially *Fusarium* mycotoxins. To the author's knowledge, no such study has been conducted on Nigerian traditional spices.

This study reports the fate of regulated *Fusarium* mycotoxins (FB_1, DON, T-2, and ZEN) in the processing steps towards the production of traditional spices (*dawadawa*, *okpehe*, and *ogiri*) and beers (*burukutu* and *pito*) from Nigeria. Moreover, other *Fusarium* mycotoxins and some modified forms namely FB_2, FB_3, $HYFB_1$, 3-ADON, 15-ADON, DON-3G, α-ZEL, β-ZEL, ZEN-14G, NIV, FUS-X, HT-2, DAS, and NEO were analysed by LC-MS/MS along the process chain of traditional spices and beers.

6.2 Materials and Methods

6.2.1 Chemicals and reagents

The sources of chemicals and reagents used for sample preparation and LC-MS/MS analysis are the same as described in chapter 3 (Section 3.2.2).

6.2.2 Sample collection

Samples of sorghum, African locust beans (ALB), African castor beans (ACB), and African mesquite beans (AMB) were purchased in October 2015. Five kilograms of sorghum was purchased from Kwoi market in Kaduna State (Nigeria) whereas 2 kg of ALB, ACB, and AMB were obtained from Onitsha market in Anambra State (Nigeria). The food samples were transported to the Laboratory of Food Analysis (Ghent University, Belgium) for further processing and analysis.

6.2.3. Processing of traditional spices (*dawadawa, okpehe*, and *ogiri*)

A laboratory-scale processing method of traditional spices namely *dawadawa, okpehe*, and *ogiri* was performed using indigenous beans: ALB, ACB, and AMB, respectively. Prior to the processing, the raw samples were analysed for the occurrence of multi-*Fusarium* mycotoxins including FB_1, FB_2, FB_3, $HYFB_1$, DON, 15-ADON, 3-ADON, DON-3G, ZEN, α-ZEL, β-ZEL, NIV, FUS-X, T-2, HT-2, DAS, and NEO. The results obtained were below the limit of detection. In order to access the effect of traditional spice processing methods on the stability of *Fusarium* mycotoxins, raw beans were spiked with FB_1, DON, T-2, and ZEN. One hundred and fifty grams of each type of bean (ALB, ACB, and AMB) was weighed and spiked with 500 µg/kg of a mixture of FB_1, DON, ZEN and T-2 toxin. Spiked beans were thoroughly homogenised, and kept in the dark for one week and analysed before proceeding with processing.

The mycotoxin concentrations in the beans (ALB, ACB, and AMB) before processing are shown in Table 6.1. Processing of the traditional spices (*dawadawa, okpehe*, and *ogiri*) was done as described in Figures 6.1 and 6.2 and samples were collected in triplicate. Spiked samples of either ALB and AMB were boiled, dehulled, and fermented for 5 days to produce *dawadawa* and *okpehe,* respectively. The same processing step was used for *ogiri* excluding dehulling, as the ACB beans obtained from the market were already dehulled (Figure 6.1).

Table 6.1. Concentration (µg/kg) of *Fusarium* mycotoxins analysed by LC-MS/MS in raw beans and sorghum spiked with 500 µg/kg and 1000 µg/kg of FB_1, DON, T-2, and ZEN, respectively

	Concentration (µg/kg)			
Sample	Deoxynivalenol	Fumonisin B_1	T-2 toxin	Zearalenone
African locust bean	362	564	492	463
Africa mesquite bean	354	429	436	430
African castor bean	363	449	400	407
Sorghum	787	806	944	721

Raw beans = African locust bean, Africa mesquite bean, and African castor bean

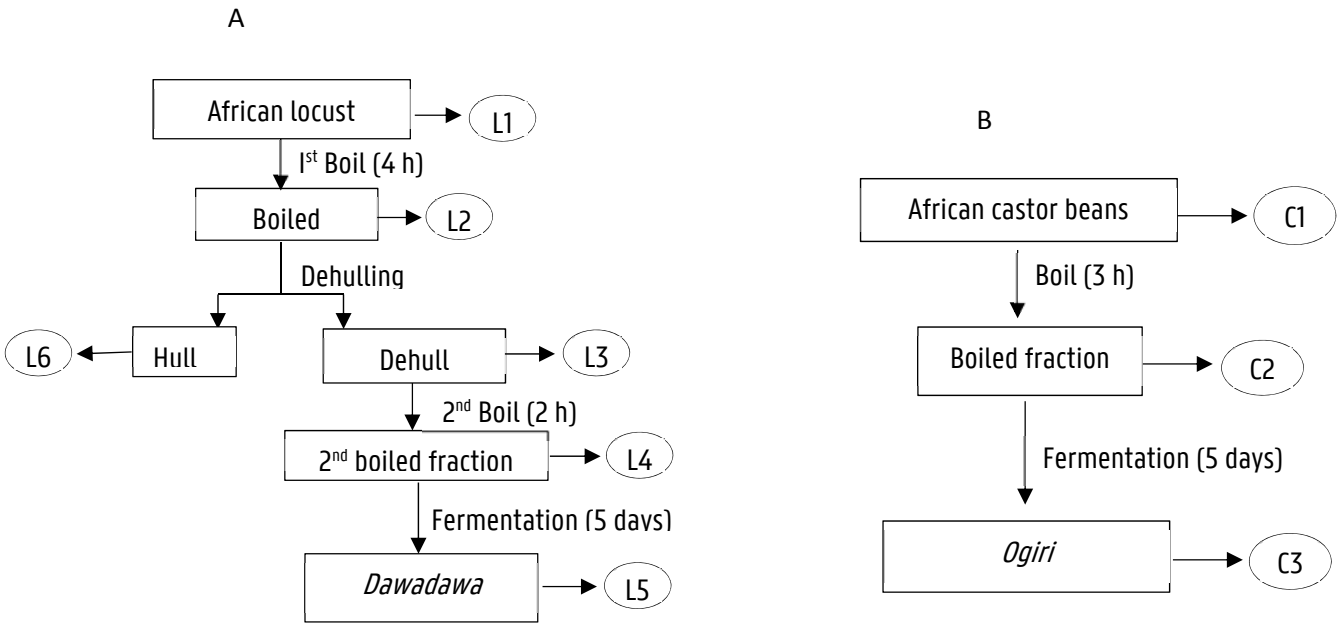

Figure 6.1 Schematic flow diagram of the traditional processing of *dawadawa* (A) and *ogiri* (B) showing the different sampling stages

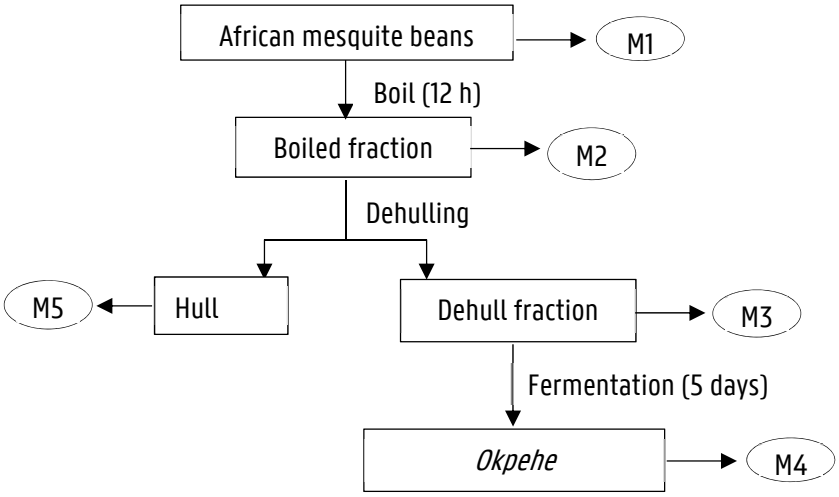

Figure 6.2 Schematic flow diagram of the traditional processing of *okpehe* showing the different sampling stages.

6.2.4. Processing of traditional beers (*burukutu* and *pito*)

Traditional beers (*burukutu* and *pito*) were processed using sorghum grains under laboratory-scale as described in Figure 6.3 (Annex 2.2: Pictorial flow diagram of *burukutu/pito* production). First, the sorghum samples were analysed for multi-*Fusarium* mycotoxins (FB_1, FB_2, FB_3, $HYFB_1$, DON, 15-ADON, 3-ADON, DON-3G, ZEN, α-ZEL, β-ZEL, NIV, FUS-X, T-2, HT-2, DAS, and NEO). Levels detected were below the limit of detection. Sorghum grains were spiked with 1,000 µg/kg of FB_1, DON, T-2, and ZEN, thoroughly homogenised, and kept in the dark for one week before processing. Prior to processing, the spiked sorghum was analysed, and the levels of FB_1, DON, ZEN, and T-2 were within the recovery rate of the analytical method used in this study (Table 6.1). The processing was done in two phases: malting and brewing. To this regard, the spiked sorghum grains were steeped in water for 2 days, and were allowed to germinate for 4 days. After germination, grains were dried at 35 °C, and milled. The milled malted sorghum was mashed with hot water, and subsequently sub-divided into 2 fractions for further processing of *pito* and *burukutu*. For the *burukutu* fraction, an adjunct (*garri*, described in Chapter 4) was added. *Pito* and *burukutu* fractions were then boiled for 2 h, filtered and allowed to ferment for 12 h. The two beer fractions were boiled for the second time, and allowed to ferment for an additional 24 h towards obtaining the final products (*pito* and *burukutu*). In addition, *pito* was further filtered to obtain a clear beer. Samples were taken in triplicate as described in Figure 6.3.

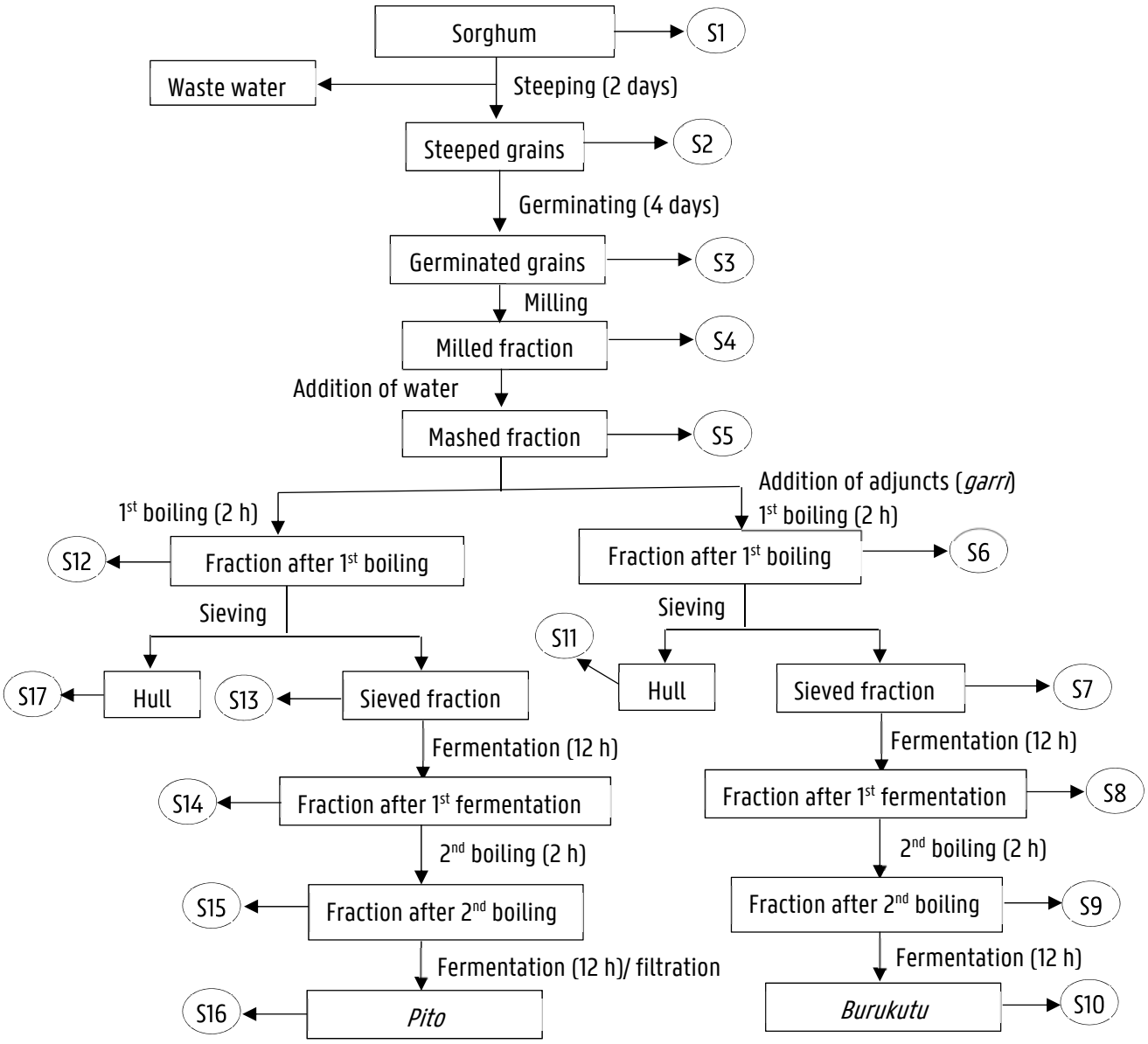

Figure 6.3 Schematic flow diagram of the traditional processing of *pito* and *burukutu* showing the different sampling stages

6.2.5. Sample preparation and clean-up

Sample preparation and clean-up of the traditional spices and beers as well as their raw materials were performed according to Monbaliu et al. [11], and are the same as described in Chapter 3 (Section 3.2.3).

6.2.6 Liquid chromatography-tandem mass spectrometry

The instrumentation and the conditions used for detection and quantification of *Fusarium* mycotoxins in both the indigenous beans and sorghum, their intermediate products, and processed products have been reported by Monbaliu et al. [12], and are the same as described in Chapter 3 (Section 3.2.6).

6.2.7. Statistical analysis

The influence of processing on the stability of *Fusarium* mycotoxins in the traditional spices and beers was performed using analysis of variance (ANOVA). Tukey's test was used as post-hoc test at a 5% level of significance to determine the significant difference between the processing steps. All statistical analyses were performed using SPSS® Version 23 software (SPSS Inc., Chicago, Illinois, USA).

6.3. Results and discussion

6.3.1. Effect of traditional processing of spices on *Fusarium* mycotoxins

The percentage of the *Fusarium* mycotoxin reduction was evaluated by comparing the initial *Fusarium* mycotoxin concentrations in the raw beans against the concentrations of the final products. A significant reduction of *Fusarium* mycotoxins (DON, ZEN, FB_1, and T-2) was observed in all the spices (*okpehe*, *ogiri*, and *dawadawa*) (Figure 6.4).

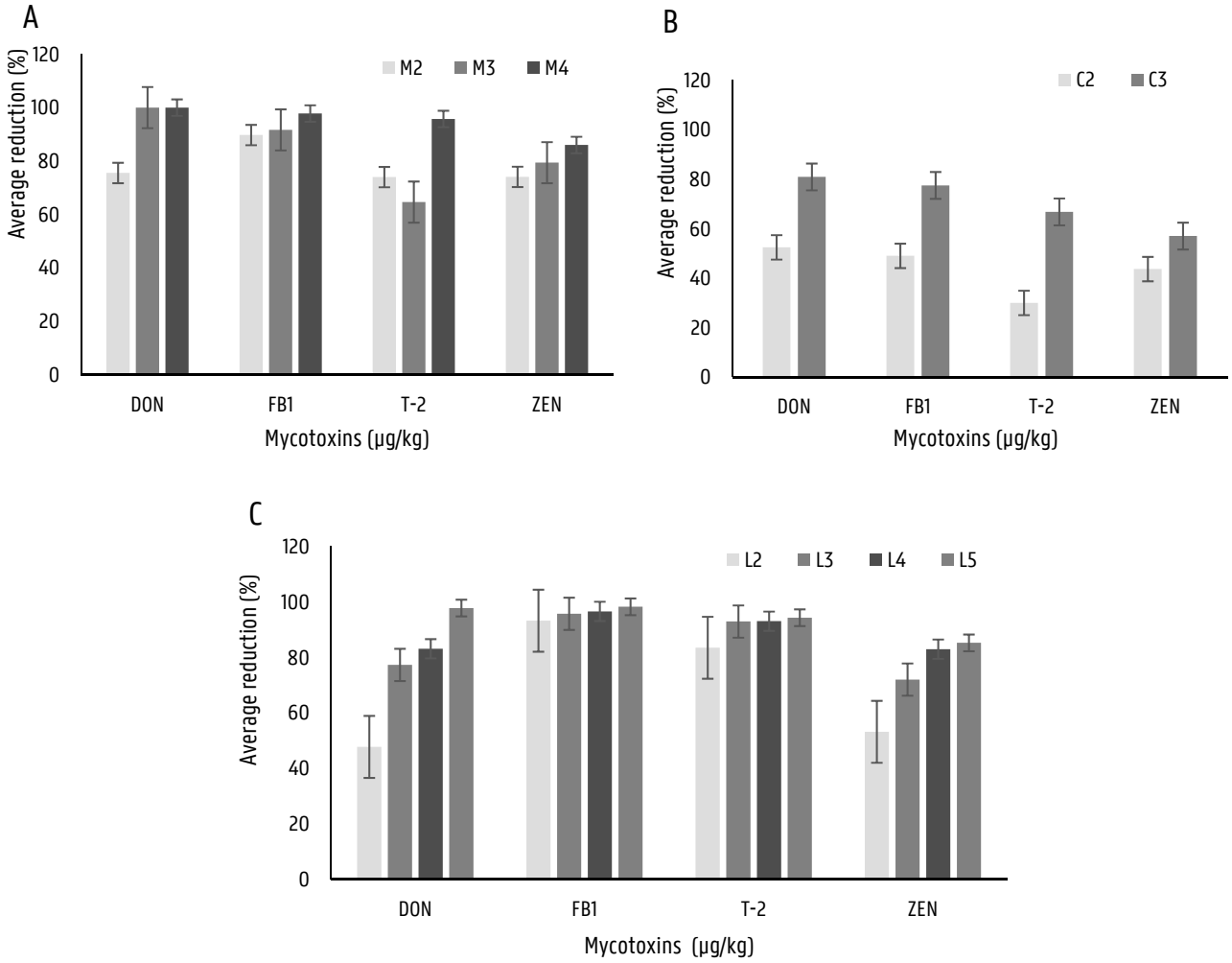

Figure 6.4. The average cumulative percentage reduction of regulated *Fusarium* mycotoxins (DON = deoxynivalenol, FB_1 = fumonisin B_1, T-2 = T-2 toxin, and ZEN = zearalenone) during processing of traditional spices with traditional beans. A = African mesquite beans: *okpehe* (M2-after boiling for 12 h, M3-after dehulling, M4-after fermentation); B = African castor beans: *ogiri* (C2-after boiling for 3h, C3-after fermentation); C = African locust beans: *dawadawa* (L2-after 1st boiling, L3-after dehulling, L4-after 2nd boiling, L5-after fermentation). Sampling was conducted in triplicate and results are presented in dry weight basis.

Boiling, which is the first processing step in the production of these spices, significantly ($p<0.05$) reduced the concentration of *Fusarium* mycotoxins irrespective of the product. This phenomenon was seen, except for the DON concentration in ALB which showed depletion (48%) after boiling, but was not statistically different ($p<0.05$). The rate at which boiling affects the mycotoxin concentration is influenced by the boiling time as well as the type of matrix. Cano-Sancho et al. [13] observed an increase in the reduction of DON with a longer boiling time which corroborates with the present study. The highest reduction of DON (76%) in the present study was observed with AMB boiled for 12 h (Figure 6.4). Similarly, Vidal et al. [14] reported the degradation (>40%) of DON during boiling of spaghetti, which they attributed to the cumulative effect of temperature, as well as leaching as they observed a significant increase of DON in the broth. A similar trend was recorded by Visconti et al. [15], who highlighted the importance of the matrix/water ratio. Although the transfer of DON to water is possible as shown by these authors, a contrary trend was observed in this study. This could be attributed to the long exposure of the toxins to heat treatment (long boiling time) as shown in Figures 6.1 and 6.2, as well as constant addition of water during the boiling process.

As for FB_1, the relative stability of this toxin at specific boiling temperatures have been described [16]. However, a 49% to 93% reduction was observed in the present study (Figure 6.4). Comparatively, a 68% - 80% reduction of FB_1 was observed by Becker-Algeri et al. [17] after applying a thermal treatment similar to domestic cooking of rice. On the other hand, heat treatment of canned and baked products at temperatures between 121 °C and 218 °C showed a significant depletion of FB_1 [18]. Regarding ZEN, 74%, 53%, and 44% of reduction was recorded after boiling AMB, ALB, and ACB for 12 h, 4 h, and 3 h, respectively. This suggests an obvious effect of the boiling time on the degradation of ZEN. It is important to mention that a longer boiling time alters the nutritional quality of food, and thus there is need to strike a balance between percentage mycotoxin degradation and the degradation of the nutritional value of these spices.

Dehulling, an indispensable processing step prior to the fermentation of traditional spices, further demonstrated a positive influence on the reduction of *Fusarium* mycotoxins. The effectiveness of dehulling in the removal of mycotoxins is dependent on the limitation of fungal colonisation and mycotoxin accumulation on the surface layer of kernels. Studies have shown the possible penetration of mycotoxins into the endosperm fraction of the grains during wet dehulling [19]. A similar distribution of mycotoxins in wheat milling fractions had earlier been reported [20]. Interestingly, this study observed a further reduction of DON, ZEN, FB_1, and T-2 at ranges between 65% and 96%. It is noteworthy to mention that the observed reduction was dependent on the type of mycotoxin and matrix (ALB and AMB) (Figure 6.4).

In addition, the concentrations of FB_1, ZEN, DON, and T-2 were further reduced after 5 days' fermentation of ALB, AMB, and ACB. A significant reduction of the toxins (excluding ZEN) was only observed for ACB. This may be attributed to the fact that the concentration of mycotoxins in ACB, prior to fermentation was higher as compared to ALB and AMB. Comparing the overall reduction of FB_1, ZEN, DON, and T-2 in the raw beans and the processed products, a higher degradation was observed during the processing of *dawadawa* and *okpehe* from ALB and AMB, respectively, when compared to processing of *ogiri* from ACB. This variation may be attributed to the exemption of the dehulling step during the processing of *ogiri*. Another possible explanation could be that the raw spiked ACB was dehulled giving room for higher absorption of the toxins. Interestingly, of all the other free and modified mycotoxins (FB_2, FB_3, $HYFB_1$, 3-ADON, 15-ADON, DON-3G, α-ZEL, β-ZEL, ZEN-14G, NIV, FUS-X, HT-2, DAS, and NEO) quantified in these products, only HT-2, one of the degradation products of T-2 was detected in the final product (*ogiri*) of ACB at 21 µg/kg.

6.3.2. Effect of traditional processing of beers on *Fusarium* mycotoxins

Fusarium mycotoxins are also important group of contaminants in fermented products especially beers, because of the unavoidable natural contamination of the raw material (cereal grains) used in the production of these products (Chapter 3). In Nigeria, sorghum serves as the major grain used in the production of fermented beverages (*burukutu* and *pito*) and a survey, previously reported in Chapter 3, revealed the co-occurrence of *Fusarium* mycotoxins in these products at varying concentrations. Due to the high consumption of this product in Nigeria, it is important to determine the behaviour of *Fusarium* mycotoxins during the processing (malting and brewing) of this product.

6.3.2.1 Malting process

The influence of the traditional malting method on regulated *Fusarium* mycotoxins (FB_1, DON, ZEN, and T-2) in sorghum samples was investigated. The result as shown in Figure 6.5. represents (a) the concentration (µg/kg) remaining after each step during malting of sorghum, and (b) the percentage reduction after each step in the processing chain. As observed, steeping resulted in a significant reduction (over 50%) of DON, FB_1, and T-2. The reduction of these toxins as observed may be due to solubilisation in water. A similar reduction of DON, T-2, and FB_1 as a result of steeping has previously been reported. Lancova et al. [4] reported over 90% reduction of DON after the steeping stage during malting of barley. A similar reduction was also reported by Habler et al. [21] and Schwarz and Howard [22]. Although there was a reduction in ZEN concentration (19%), this was not significant as compared to the other toxins. The findings are in contrast with an earlier study by Kocic-Tanackoc et al. [23],

who reported an increase in the ZEN concentration as a result of steeping. Apart from FB_1, ZEN, DON, and T-2, no other *Fusarium* mycotoxins were detected after steeping.

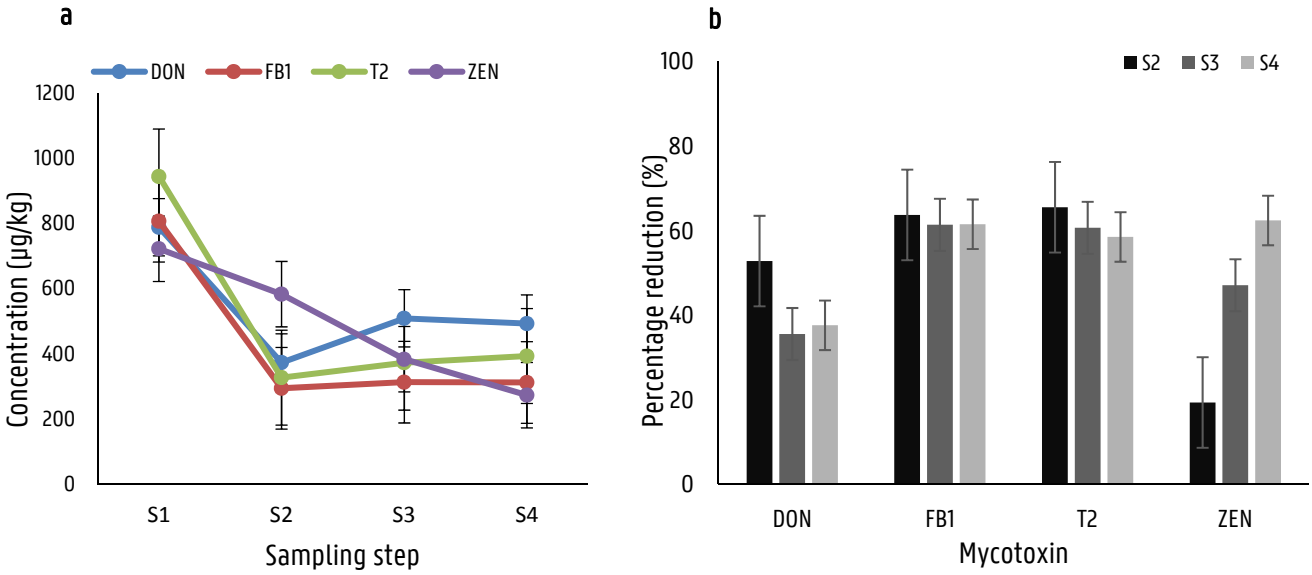

Figure 6.5. (a) *Fusarium* mycotoxin concentration (µg/kg) remaining after each step during malting of sorghum, (b) average cumulative percentage reduction of regulated *Fusarium* mycotoxins during malting of sorghum. S1 - spiked sorghum, S2 – after steeping, S3 – after germination, S4 – after drying/milling. Sampling was performed in triplicate and results are presented in dry weight basis.

After 4 days of germination, the level of ZEN further decreased, in contrast to the trend observed for DON, FB_1, and T-2. A similar increase and decrease in DON and ZEN, respectively, during germination has been reported [4,21,23]. It is important to highlight that additional mycotoxins including DON-3G (39 µg/kg), 15-ADON (17 µg/kg), α-ZEL (24 µg/kg), and HT-2 (36 µg/kg) were detected after the germination stage. This may be as a result of an increase in enzymatic activities of the germinated sorghum kernels, thus leading to the release of these compounds. In addition, it is possible that there was accumulation of *Fusarium* fungi during this process, hence producing toxins. After germination, malted grains are dried (kilning), and cleaned to remove the rootlet fraction. This act has been reported to significantly reduce the mycotoxin concentration [4]. No significant changes were observed in the present study after drying and milling of malted sorghum, probably because the malted grains used for the production of traditional beers were not cleaned.

6.3.2.2 Brewing process

Unlike the conventional beer production that requires additional ingredients such as hops and yeast, the ingredients needed to produce Nigerian traditional beers (*burukutu* and *pito*) include malted sorghum, water, and a starter from the previous brew to initiate fermentation. Depending on the type of beer, an adjunct (*garri*) may be added as in the case of *burukutu*. In this study, raw sorghum grains were used to produce *burukutu* and *pito* (Figure 6.3). Due to the similarity in the processing methods for the two beers, no significant difference was observed between the two beers, and as such the result of only *burukutu* will be presented in this Chapter. In addition to the spiked mycotoxins, other *Fusarium* mycotoxins were also analysed. The results were calculated using the mass transfer method previously described for calculation of *Fusarium* mycotoxins during the production of beer by Lancova et al. [4], and are presented in Figure 6.6 and Table 6.2. This method converts the concentration value of the *Fusarium* mycotoxins detected to the total amount of mycotoxin based on the total weight or volume of a sample. To further clarify, the starting raw material (malted sorghum: 0.5 kg), adjunct (*garri*: 0.2 kg) and 4.2 litres of water were used. Considering the concentration of DON in the malted sorghum which equals to 491 µg/kg (Figure 6.6a, sampling step S4), the total amount of DON in 0.5 kg is approximately 246 µg (Figure 6.6b, Sampling step S4). In employing these values, the total amount of DON in the first brewing intermediate (S5: mashed fraction) is equal to 506 µg (4.2 l of water + 0.2 kg of adjunct * 115 µg/kg DON concentration).

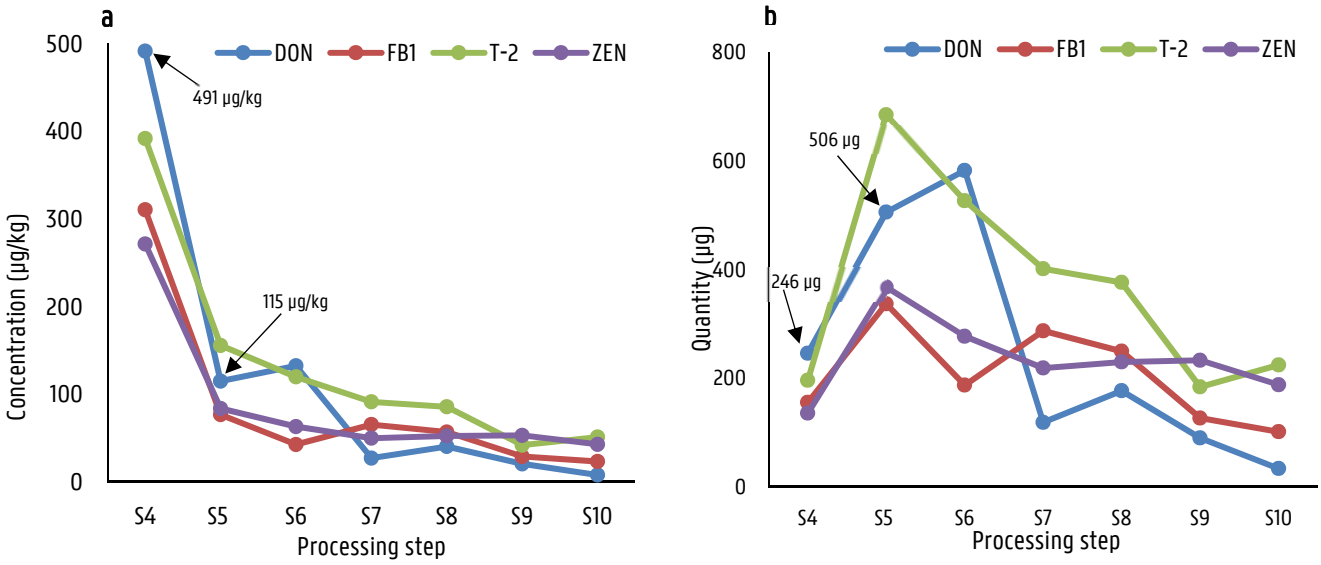

Figure 6.6. (a) *Fusarium* mycotoxin concentration (µg/kg) and (b) total amount (µg) of *Fusarium* mycotoxins remaining after each step during brewing of *burukutu*. S4 – milled malted fraction, S5 -mashed fraction, S6 – fraction after 1st boiling, S7 – sieved fraction, S8 – fraction after 1st fermentation, S9 – fraction after 2nd boiling, S10 – fraction after 2nd fermentation (*burukutu*).

As observed in Figure 6.6a, the concentrations of all the mycotoxins were significantly reduced upon addition of water to the milled malted sorghum (mash fraction). However, a contrast trend was observed when the mass transfer method was applied (Figure 6.6b). DON, T-2, FB_1, and ZEN increased by 106%, 250%, 117%, and 170% in the mashed fraction, respectively, as compared to the initial values detected in the malted grains.

Table 6.2 Levels of modified *Fusarium* mycotoxins detected along the process chain of traditional beer (*burukutu*)

Sample code	Sample description	Concentration (µg/kg)				
		DON-3G	15-ADON	α-ZEL	β-ZEL	HT-2
S4	Milled malted fraction	39±18	17±9.8	24±21	nd	36±13
S5	Mashed fraction	nd	nd	nd	nd	nd
S6	Fraction after 1st boiling	nd	nd	nd	nd	nd
S7	Sieved fraction	nd	nd	nd	nd	nd
S8	Fraction after 1st fermentation	16±15	<LOQ	<LOQ	<LOQ	nd
S9	Fraction after 2nd boiling	23±20	14±5.3	18±13	24±21	nd
S10	Fraction after 2nd fermentation	26±11	16±7.7	22±18	31±16	nd

DON-3G = deoxynivalenol-3-glucoside, 15-ADON = 15-acety-deoxynivalenol, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, nd = not detected, LOQ = limit of quantification

A similar increase of DON and its acetylated and glycosylated metabolites during mashing has been reported [4,22,24]. In addition, the related compounds (DON-3G, 15-ADON, α-ZEL, and HT-2) previously detected in the malted grains were completely reduced below the limit of detection after the addition of water (mashing). FB_1 was constantly reduced in subsequent processing steps including 1st boiling, sieving, 1st fermentation, 2nd boiling, and 2nd fermentation. In contrast, an increase in DON, ZEN, and T-2 concentration was observed after 1st boiling 1st fermentation, and 2nd fermentation, respectively (Figure 6.6). The increase in DON and ZEN may be attributed to the release of these compounds from the matrix [25]. Ironically, metabolites (DON-3G, 15-ADON, α-ZEL, and β-ZEL) of these compounds were further detected after the 1st fermentation, and increased subsequently along the process chain. Lancova et al. [4] reported the possible metabolisation of ZEN to β-ZEL by *S. cerevisiae* during beer production. ZEN-14G, $HYFB_1$, and 3-ADON were not detected along the process chain. A comparison of the initial concentration of malt and the final beer revealed an appreciable reduction of DON (86%) and FB_1 (35%). Lancova et al. [4] also reported a decrease (30%) of DON and ZEN as a result of brewing. Contrary to the report of these authors, T-2 (15%) and ZEN (38%) detected in the final products were higher as compared to the concentration of the malt.

6.4. Conclusion

The effect of processing methods used for the production of traditional spices and beers on *Fusarium* mycotoxins was evaluated. The results revealed that the influence of processing on mycotoxins is dependent on the processing methods as well as the process parameters. For the traditional spices, the concentrations of these toxins in the final products (*dawadawa*: FB_1 - 10±18, DON - 8±7, T-2 - 28±14, ZEN - 69±16 µg/kg; *okpehe*: FB_1 - 9±7, DON - nd, T-2 - 19±10, ZEN - 60±21 µg/kg; *ogiri*: FB_1 - 101±44, DON - 70±17, T-2 - 133±24, ZEN - 174±48 µg/kg) were significantly reduced, while one degradation compound (HT-2 - 21±3.3 µg/kg) was detected. A reverse trend was observed in respect to *burukutu* beer with T-2, exceeding the levels detected in the fraction after 2nd boiling (T-2 – 22%). It is worrisome that a number of toxins, which were initially not detected, were found in the final product suggesting possible modification of free mycotoxins, or release of bound toxins upon processing (boiling and fermentation). Although there was a reduction of mycotoxins during processing of traditional spices and beer, it is important to mention that a reasonable concentration of these toxins (FB_1 - 111±65, DON - 37±5.8, T-2 - 245±63, ZEN - 222±31 µg/kg) was detected in the final products. Considering that traditional beers are consumed at large quantities because of their low alcohol content and affordability (when compared with the commercial bottled/canned beers), this may be a source of mycotoxin exposure to consumers. Thus, a good quality of raw materials is paramount for obtaining a mycotoxin-free processed product. The influence of processing on Nigerian traditional infant foods will be discussed subsequently in Chapter 7.

6.5. References

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INFLUENCE OF PROCESSING METHODS OF NIGERIAN TRADITIONAL INFANT FOODS (*OGI* AND SOYBEAN POWDER) ON *FUSARIUM* MYCOTOXINS

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CHAPTER 7: INFLUENCE OF PROCESSING METHODS OF NIGERIAN TRADITIONAL INFANT FOODS (*OGI* AND SOYBEAN POWDER) ON *FUSARIUM* MYCOTOXINS

7.1. Introduction

Infants and young children are most vulnerable to food contaminants, especially mycotoxins. This is because of their lower body mass, higher metabolic rate, immature organ functions and detoxication mechanisms in combination with a high consumption rate of more restricted diets [1]. Several diseases ranging from acute to chronic caused by mycotoxins have earlier been described in Chapter 1. In addition, the IARC recently reported the possible association between mycotoxins (FBs) and stunting in children [2]. In view of these facts, stringent regulatory standards are put in place in the developed countries, thus conferring relative protection of infants and young children from exposure to these secondary fungal metabolites. Unfortunately, the reverse is the case in developing countries, sited in SSA. The situation is made worse by the feeding pattern and food culture of this age group which is heavily dependent on the cereals and other agricultural commodities that are susceptible to fungi and mycotoxin contamination.

Ogi, as briefly described in Chapter 3, is the most preferred food for infants and young children in Nigeria, probably because of its affordability to both low and high-income earners, its ease of preparation, multi-dietary purposes, and multi-nutritional benefits. It is a non-alcoholic starchy food that can be produced either from maize, sorghum, or millet, however, maize is the most preferred cereal because of its ease in cultivation and price. *Ogi* is produced by submerge fermentation of cereal grains for two to three days, immediately followed by wet milling and sieving through a fine mesh. The fermentation process of *ogi* is initiated by chance inoculation under uncontrolled environmental conditions, thus resulting to a product (*ogi*) of variable quality [3]. Although *ogi* provides high calories, studies have shown its low protein content [4–6], thus it is nutritionally inadequate especially as a weaning food [7,8]. In order to boost the nutritional quality of *ogi*, it is often complemented with legumes products such as processed soybean powder [9–11].

Soybean powder is the most common food product of soybeans (*Glycine max*) in Nigeria. It is produced either by roasting beans and then milling into powder or by blanching, sun-drying, and milling of the beans. Soybean, known for its high protein content presents a healthy formulation complement of infant meals towards combating malnutrition. Notwithstanding the importance of *ogi* and processed soybean powder (PSP) in the diet of infants and young children, consumption of these products could be a potential route of multi-mycotoxin exposure as revealed

in the previous Chapters. As observed, a wide range of *Fusarium* mycotoxins were detected in these processed products with some of the samples containing a cocktail of contaminants (Chapters 3 and 5). This trend of contamination in processed food products has also been reported by several authors across SSA [12–16].

In the quest to reduce human exposure to mycotoxins, efforts have been channelled towards understanding the behaviour of mycotoxins during food processing. So far, several studies have elucidated the behaviour of mycotoxins especially *Fusarium* mycotoxins during food processing in the developed world [17–22], however, this trend is in contrast with the state of research in developing countries including Nigeria. Very little information is known about the behaviour of *Fusarium* mycotoxins during processing of traditional foods from Nigeria. The available studies focused on the degradation of the free *Fusarium* mycotoxins without putting into consideration the possible transformation of free toxins to their modified forms. In this light, and coupled with the fact that traditional processing is done with rudimentary methods under uncontrolled environments, it is therefore imperative to conduct studies that can unravel the effect of traditional processing methods on *Fusarium* mycotoxins contamination of *ogi* and processed soybean powder.

The current study aimed to investigate the fate of *Fusarium* mycotoxins (FB_1, ZEN, DON, and T-2) during the processing of *ogi* and soybean powder used as infant foods in Nigeria. In addition, other *Fusarium* mycotoxins including the modified mycotoxins (FB_2, FB_3, $HYFB_1$, 3-ADON, 15-ADON, DON-3G, α-ZEL, β-ZEL, ZEN-14G, NIV, FUS-X, HT-2, DAS, and NEO) were investigated along the processing chain of *ogi* and processed soybean powder.

7.2. Materials and methods

7.2.1. Chemicals and reagents

All chemicals and reagents used for sample preparation and LC-MS/MS analysis were of analytical grade and their sources and preparations are the same as described in Chapter 3 (section 3.2.2).

7.2.2 Origin of maize and processing of *ogi*

Maize grains were obtained from a market in Kaduna town (Nigeria), and the grains were naturally contaminated with 3,531 µg/kg of FBs (FB_1 – 3,239 µg/kg, FB_2 – 241 µg/kg, FB_3 – 51 µg/kg), 99 µg/kg of DON, 68 µg/kg of ZEN, 24 µg/kg of T-2, and 337 µg/kg of $HYFB_1$ (Table 7.1) (Annex 2.3: Pictorial flow diagram of *ogi* production). The maize was processed into *ogi* using the indigenous method. Samples were collected along the processing chain as shown in

Figure 7.1. Briefly, maize grains were first cleaned by winnowing, thoroughly homogenised, and sub-divided into three batches. Each batch (500 g) of the maize grains was steeped in clean water to the ratio of 1:3 (maize:water) in a plastic bowl, and allowed to ferment spontaneously at ambient temperature for 3 days (72 hr). After the 3 days of fermentation, the water was drained, the maize grains were milled, and sieved through a fine mesh using water to separate the starch and the hull (chaff). The starch component - *ogi* was pressed with a jute bag to remove the excess water. The process was done in triplicate. Samples were collected as shown in Figure 7.1, and were stored at -20 °C until analysis.

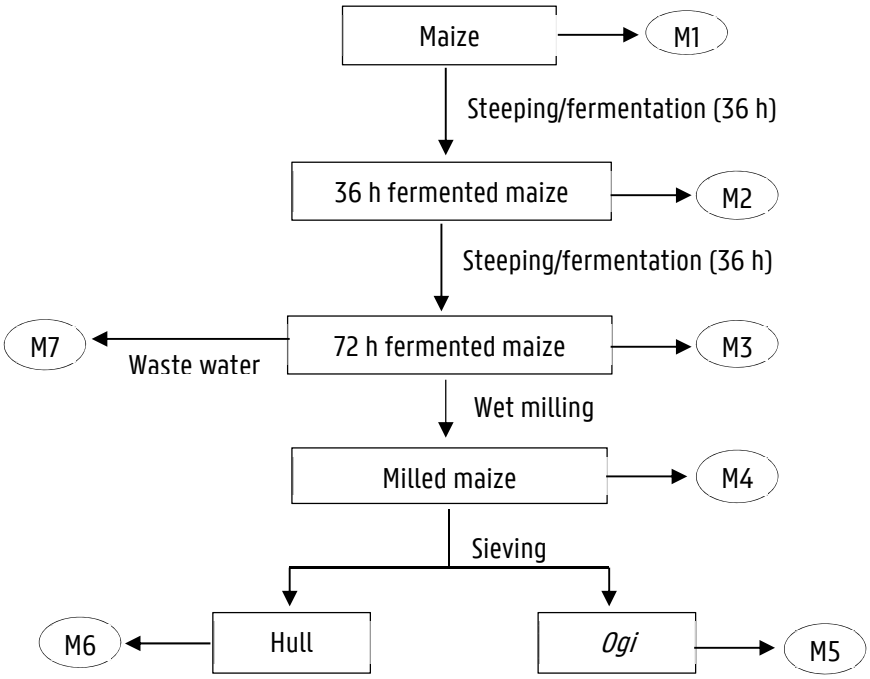

Figure 7.1. Schematic flow diagram of the production process of *ogi* showing the different sampling points (M)

7.2.3. Origin of soybean and processing of soybean powder

Soybean samples obtained from a market in Makurdi town (Benue State, Nigeria) were naturally contaminated with 139 µg/kg of FBs (FB_1 – 52 µg/kg, FB_2 – 48 µg/kg, FB_3 – 39 µg/kg), 30 µg/kg of ZEN, and 23 µg/kg of T-2. The soybean samples were processed into PSP using two different indigenous methods use in Nigeria (Figure 7.2) (Annex 2.4: Pictorial flow diagram of soybean powder production). In brief, 3 kg of soybeans were sorted to remove dirt. It was

thoroughly mixed and subsequently divided into 6 parts each weighing 500 g. In the first method, soybeans were dry-roasted in a pan, dehulled, and milled into PSP. In the second method, soybeans were soaked, blanched, dehulled, and washed, and then sun dried, and milled into PSP. Samples were collected along the processing chain as shown in Figure 7.2, and stored at -20 °C until analysis

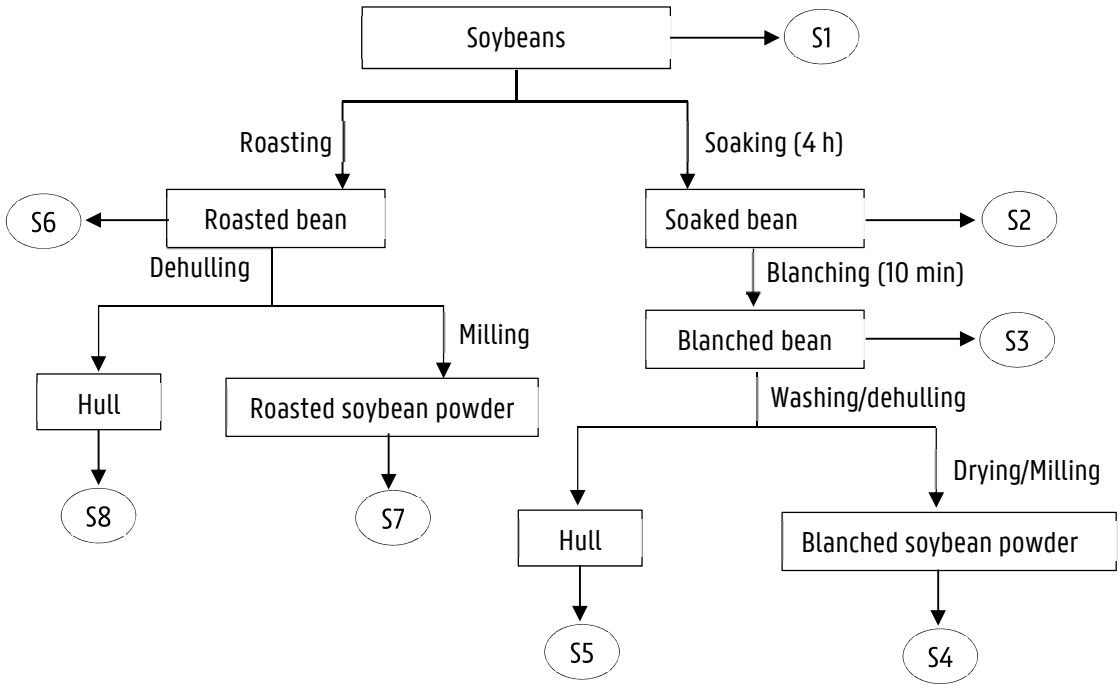

Figure 7.2. Schematic flow diagram of the production process of soybean using two different methods, and showing the different sampling points (S)

7.2.4. Laboratory-scale production process of *ogi* and soybean powder with spiked maize and soybean grains

Also, a laboratory-scale experiments were performed using spiked maize and soybean to determine the overall effect of the traditional processing methods used to produce *ogi* and PSP on four regulated *Fusarium* mycotoxins (FB_1, DON, ZEN, and T-2). Briefly, cleaned and sorted maize and soybean were thoroughly homogenised, and representative samples were taken from the lots and analysed for the natural occurrence of the *Fusarium* mycotoxins and possible metabolites (FB_1, FB_2, FB_3, $HYFB_1$, DON, 3-ADON, 15-ADON, DON-3G, ZEN, α-ZEL, β-ZEL, ZEN-14G, T-2, HT-2, NIV, FUS-X, DAS, NEO). The result showed that mycotoxin concentrations were below the limit of

detection recorded in this method (Chapters 3 and 5). Processing of *ogi* and PSP were carried out in triplicate using maize and soybean artificially contaminated by spiking with 1000 µg/kg and 500 µg/kg mycotoxin (FB_1, DON, ZEN, and T-2) mix. The spiking levels were within the linear range of the calibration curve. Prior to the preparation of *ogi* and PSP, spiked samples were analysed and the levels were consistent to the recovery levels recorded for this method. Processing of *ogi* and PSP were done as described in Section 7.2.2 (Figure 7.1) and Section 7.2.3 (Figure 7.2), respectively.

7.2.5. Sample extraction and mycotoxin analysis

Sample preparation and extraction were performed according to Monbaliu et al. [23], and are described in Chapter 3 (Section 3.2.3). The instrumentation and the conditions used for detection and quantification of *Fusarium* mycotoxins in maize and soybeans, their intermediate products, and processed products have be reported by Monbaliu et al. [24], and are the same as described in Chapter 3 (Section 3.2.6).

7.2.6. Statistical analysis

Basic descriptive analysis was performed using Microsoft office Excel 2007 (Redmond, WA, USA) and the statistical analyses were performed using SPSS® Version 23 software (SPSS Inc., Chicago, Illinois, USA). The influence of processing on the mycotoxin stability in *ogi* and PSP was performed by analysis of variance (ANOVA). Tukey's test was used as post-hoc test at 5% level of significance.

7.3. Results and discussions

7.3.1. Influence of the *ogi* processing method on *Fusarium* mycotoxins

The effect of different processing steps on *Fusarium* mycotoxins during *ogi* production was studied using naturally contaminated maize grains, and artificially contaminated maize grains in a laboratory-scale setting. In total, 7 toxins were detected in the naturally-contaminated maize as shown in Table 7.1. Generally, there was a significant reduction (range: 58±2.8% (SD) – 100%) of all mycotoxins when compared to the initial concentration of the raw maize. Reduction in mycotoxin concentration started immediately after 36 h of fermentation for FB_1, FB_2, FB_3, DON, ZEN, and T-2, except for $HYFB_1$ which had a slight increase (16±34% (SD)) at this stage (Table 7.1). The increase observed for $HYFB_1$ may be attributed to microbial degradation of FB_1 to $HYFB_1$ as previously revealed by Heinl et al. [25] and Grenier et al. [26]. A notable percentage degradation was also recorded for the toxins after wet milling (M4), with up to a 100% reduction for FB_3, ZEN, and T-2, while the level of DON detected was below limit of quantification. Although, a 70% reduction of FB_1 was recorded between the raw maize and the final product, a relatively high concentration (958±2.8 µg/kg) of this toxin was detected in the final product, which corresponded with the initial high concentration detected in the raw maize.

Table 7.1. *Fusarium* mycotoxin concentration (µg/kg) in processing intermediates and products collected during *ogi* production with naturally-contaminated maize

Samples	Sample codes	*Fusarium* mycotoxin concentration (µg/kg)						
		FB_1	$HYFB_1$	FB_2	FB_3	DON	ZEN	T-2
Maize	M1	3239*	337*	241*	51*	99*	68*	24*
36 h fermented maize	M2	2280±63	390±34	172±32	<LOQ	61±6	37±4	nd
72 h fermented maize	M3	2171±36	319±33	153±11	nd	33±3	28±4	nd
Milled maize	M4	869±45	276±15	128±22	nd	<LOQ	nd	nd
Ogi	M5	958±3	141±23	33±6	nd	nd	nd	nd
Hull	M6	1023±57	89±4	68±4	nd	nd	nd	nd

oncentrations were calculated on dry weight basis. Sample codes refer to sampling points as shown in Figure 7.1. FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; $HYFB_1$ = hydrolysed fumonisin B_1; DON = deoxynivalenol; ZEN = zearalenone; T-2 = T-2 toxin; nd = not detected, LOQ = limit of quantification. Experiment were performed in triplicate. *Concentration = there was no deviation in the values of the triplicate.

To further elucidate the effect of the *ogi* production process on *Fusarium* mycotoxins, a laboratory-scale *ogi* process was carried out using maize grains spiked at two concentrations (1,000 µg/kg and 500 µg/kg) of FB_1, DON, ZEN, and

T-2. The observed reduction of *Fusarium* mycotoxin concentrations ranged between 65%±34 (SD)- 83%±8.2 (SD) irrespective of the mycotoxin types and initial concentration of the raw maize (Figure 7.3 and 7.4).

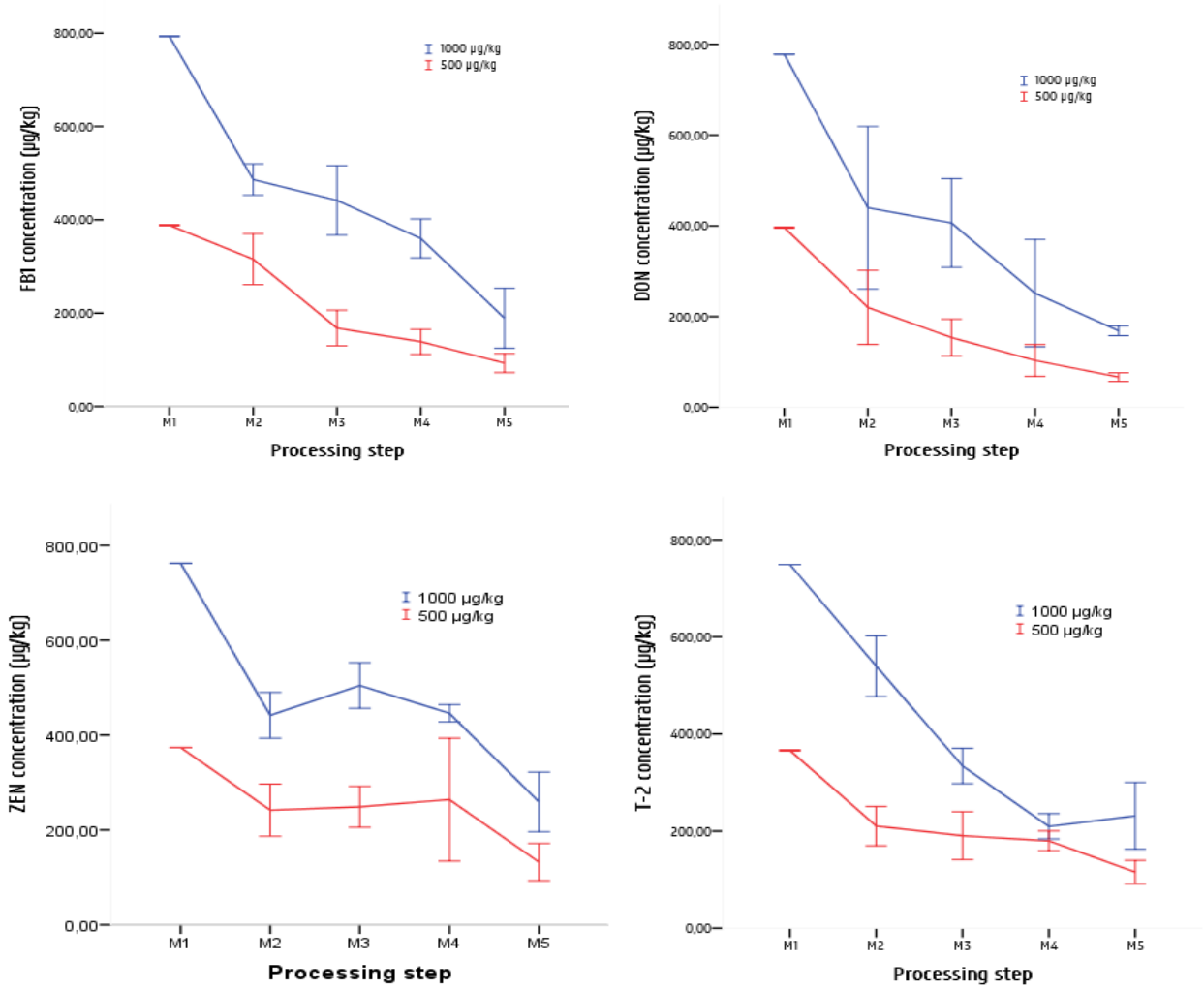

Figure 7.3. Concentrations (µg/kg) of fumonisin B_1 (FB_1), deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2) in the different processing steps of *ogi* (M1 = maize, M2 = 36 h fermented maize, M3 = 72 h fermented maize, M4 = milled maize, M5 = *ogi*). Concentrations were calculated on dry weight basis. Experiments were performed in triplicate.

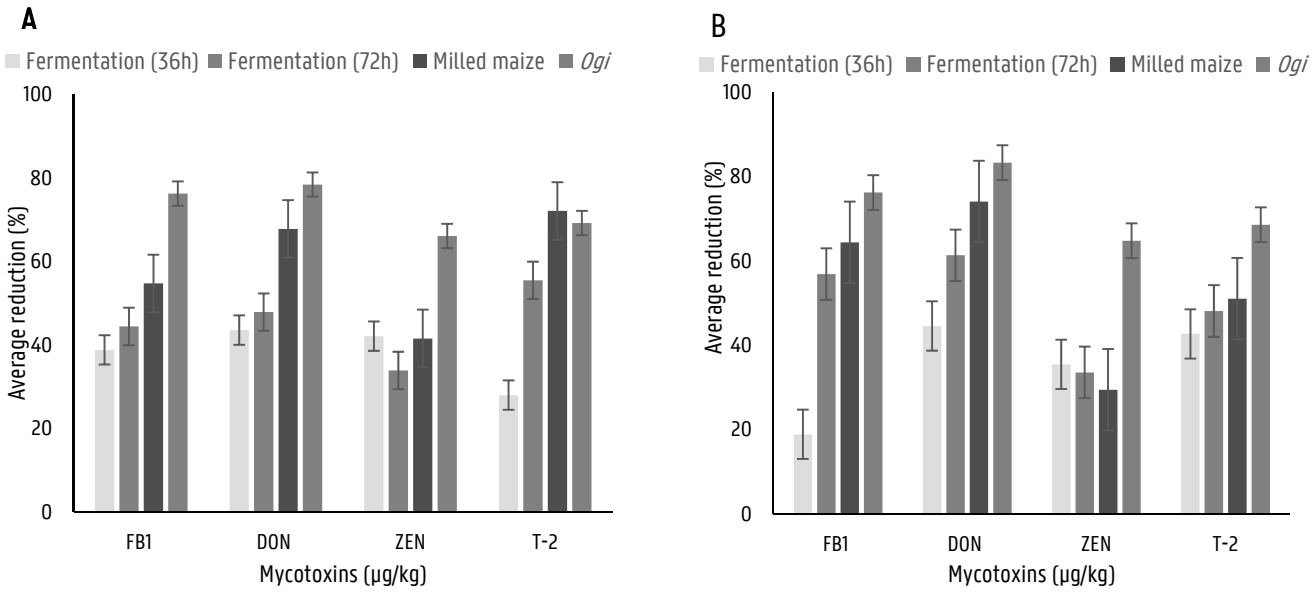

Figure 7.4. Average cumulative percentage reduction of regulated *Fusarium* mycotoxins (FB_1 = fumonisin B_1, DON = deoxynivalenol, ZEN = zearalenone, and T-2 = T-2 toxin) during processing of *ogi* with spiked maize grains (A: 1,000 µg/kg, B: 500 µg/kg). Experiments were performed in triplicate.

Among the four mycotoxins, DON was observed to have the highest reduction rate in the final product which ranged between 78%±30 (SD) and 83%±8.2 (SD), depending on the concentration of the initial maize grains, and was succeeded by FB_1 (76%) (Figure 7.4). After 72 hours of fermentation, the DON concentration was reduced by 48%±85 (SD) and 61%±35 (SD), while T-2 had a reduction rate ranging between 48%±91 (SD) and 55%±160 (SD) (Figure 7.4). Trichothecenes are classified as water-soluble compounds thus expected to leach into water, although, the extent of leaching is dependent on the initial concentration and ratio of contaminated grains to water. The degradation of DON and T-2 during *ogi* processing may be attributed to multiple factors such as leaching. Steeping of grains in water has shown a possible reduction of DON between 30% and 49% [27]. Collins and Rosen [28] reported a transfer of approximately 67% of T-2 initially present in maize to steep water. An earlier study by Lancova et al. [29] reported a similar trend as a result of steeping, which led to DON reduction below 10% of the original concentration. Similarly, Visconti et al. [30] and Vidal et al. [21] reported the potential of DON leaching into water when cooking spaghetti, however, the degree of leaching is commensurate to the ratio of water [30].

Although it is difficult to distinguish the individual effect of steeping and fermentation during the production of *ogi*, studies have reported the degradation of TH especially DON during production of traditionally fermented beverages

[15,31]. Using a post-hoc test, the author observed no significant reduction ($p < 0.05$) of DON across the fermentation time interval (36 h - 72 h), irrespective of the initial concentration. In addition, studies have revealed the possible formation of glucosylated DON (DON-3G). Lancova et al. [29] observed a significant increase of DON-3G as well as acetylated DON during the malting and brewing process. Remarkably, bio-transformation products of DON (DON-3G and 3-ADON), which were initially absent in the raw maize sample were detected after 72 h fermentation in the order of DON-3G > 3-ADON, and at concentrations of 16±3.2 µg/kg and 9±5.5 µg/kg in the final *ogi* produced with 1,000 µg/kg spiked maize grains, respectively (Table 7.2). The levels of DON-3G and 3-ADON detected in the *ogi* produced with 500 µg/kg spiked maize grains were below the LOQ. The increase of DON-3G as a result of fermentation during bread making has also been reported [18,20,32]. The same trend was observed with T-2 and its degradation product (HT-2). HT-2 was detected in the product although at low concentrations (Table 7.2). Evidence of these compounds contaminating *ogi* was earlier shown in Chapter 3.

Table 7.2 Concentrations (µg/kg) of *Fusarium* mycotoxins detected in process intermediates and final products during the *ogi* production process

Samples	Sample codes	*Fusarium* mycotoxins (µg/kg)								
		FB_1	DON	ZEN	T-2	3-ADON	DON-3G	$HYFB_1$	HT-2	β-ZEL
Maize[a]	M1	794*	778*	762*	749*	nd	nd	<LOQ	nd	nd
Fermentation (72 h)	M3	442±64	406±85	505±42	334±160	22±10	57±7	121±25	36±20	86±46
Ogi	M5	189±56	169±30	259±55	231±110	9±6	16±3	31±10	29±11	67±28
Hull	M6	75±27	115±20	230±58	109±44	11±5	14±8	16±17	24±9	25±11
Waste water	M7	155±22	308±15	64±16	110±46	<LOQ	18±3	54±18	15±6	10±9
Maize[b]	M1	388*	396*	374*	366*	nd	nd	<LOQ	nd	nd
Fermentation (72 h)	M3	168±33	154±35	249±38	190±91	<LOQ	18±7	96±53	13±9	66±19
Ogi	M5	93±18	67±8	132±34	116±49	<LOQ	<LOQ	<LOQ	12±12	16±4
Hull	M6	75±21	39±3	102±37	63±31	8±6	nd	14±13	<LOQ	11±8
Waste water	M7	129±51	175±66	52±21	85±50	nd	<LOQ	63±29	<LOQ	< LOQ

Maize[a] = spiked with 1000 µg/kg; Maize[b] = spiked with 500 µg/kg; FB_1 = fumonisin B_1; DON = deoxynivalenol; ZEN = zearalenone; T-2 = T-2 toxin, 3-ADON = 3-acetyl-deoxynivalenol; DON-3G = deoxynivalenol-3-glucoside; $HYFB_1$ = hydrolysed fumonisins; HT-2 = HT-2 toxin; ß-ZEL = ß-zearalenol; <LOQ = less than the limit of quantification; nd = not detected. Sample codes refer to sampling points as shown in Figure 7.1. Values are the average of a triplicate study. *Concentration = there was no deviation in the values of the triplicate. Concentrations were calculated on dry weight basis.

After 72 h submerged fermentation of maize grains, a significant reduction of FB_1 was observed, ranging from 44% to 57% depending on the initial concentration of maize grains (Figure 7.4). A significant ($p < 0.05$) difference was

recorded between 36 h and 72 h fermentation in the *ogi* process initiated with maize grains spiked at lower concentrations (500 µg/kg), whereas an opposite trend was observed with *ogi* processed with higher FB_1 contaminated maize grains (spiked with 1,000 µg/kg). This result could be attributed to the fact that steeping water may have been saturated, since the same ratio of maize:water was used for both experiments. FB_1 is able to migrate from kernels into steeping water because of the high polarity of this compound [33], consequently FB_1 (range: 129±51 µg/kg – 155±22 µg/kg) was detected in the waste water used for fermentation. In addition to the degradation effect caused by leaching, the activities of micro-organisms during the fermentation process should not be neglected. Reduction of FBs as a result of fermentation has been reported by several authors [14,34,35] and also observed in Chapter 6. Fermentation during *ogi* production is a spontaneous (natural) process which is usually influenced by the dominating micro-organisms in the environment, however, studies on the elucidation of microbial diversity during the production of *ogi* have reported lactic acid bacteria (LAB) as the dominant organism [36–39]. A significant reduction of FB_1 ranging from 56% to 67% as a result of LAB fermentation was reported by Mokoena et al. [34]. Okeke et al. [14] also reported a similar trend during *ogi* processing, however, at a higher reduction rate (66% - 89%). The slight variation in reduction rate observed in the present study as compared to these authors could be attributed to many factors such as the different processing conditions (ratio- water: maize), the initial mycotoxin concentration as well as the environment. It is hypothesised that the environment plays a major role in the microbiological quality of *ogi,* because of its influence on the microbial habitation and activities during *ogi* processing. Wet milling using the Braun Minipimer blender further reduced FB_1 to 55% - 64% (Figure 7.3 and 7.4). Upon separation of the maize hull from the starch (*Ogi*), a further reduction of FB_1 up to 76% was observed. Previous studies highlighted the deposition of mycotoxins on the outer layers of grains, thus leading to reduction of mycotoxins upon separation of the hull through dry milling [19,40]. In contrast, more deposition of FB_1 in *ogi* was observed as compared to the hull (Table 7.2). This may be attributed to the traditional separation method which involved the washing of the hull on a tiny pore sieve, while the starch components go through the sieve. In addition to FB_1, a reasonable amount of $HYFB_1$ was detected in the samples along the processing chain (Table 7.2). A similar report on the increase of $HYFB_1$ along the production chain of indigenous fermented non-alcoholic maize beverage (*kunu-zaki*) from Nigeria has been stated [15].

In contrast to the high reduction (>80%) of ZEN after fermentation recorded by Okeke et al. [14] during *ogi* processing, a 34% decrease of ZEN was observed after 72 h fermentation in the present study regardless of the initial concentration. There was no significance difference ($p < 0.05$) across the fermentation stages (36 h and 72

h). Okeke et al. [14] ascribed the degradation of ZEN in their study to mostly the activities of non-LAB species, specifically *Bacillus subtilis,* which were earlier implicated to degrade ZEN in liquid medium by Cho et al. [41]. In addition, ZEN may bind to *Saccharomyces cerevisiae*, as well as LAB [35,41]. Zhao et al. [42] studied the efficiency of three strains of *Lactobacillus plantarum* in detoxifying ZEN in fermented foods, and their result revealed a similar reduction trend observed in this study. Although, bacterial profiling was outside the scope of the present study, the binding affinity of ZEN is dependent on the viability of the bacteria and the type of strains, incubation temperature, as well as the initial concentration of the mycotoxins [42]. In addition, fermentation may lead to the hydrolysis of ZEN to its metabolites [43]. β-ZEL was detected in the intermediate, as well as the final products, irrespective of the starting concentration, while ZEN-14G and α-ZEL were absent. The same trend was reported by Mizutani et al. [43] and Ezekiel et al. [15] during the beer fermentation process suggesting that fermentation could be a possible way to detoxify ZEN. In addition to the reduction caused by steeping/fermentation, a further reduction was observed after wet milling and removal of hull (Figures 7.3 and 7.4).

Finally, there was a significant reduction in all the mycotoxins when the initial concentration and the final product were compared. It is worth highlighting that the sum of toxins detected in the final fractions (*ogi*, hull, and fermentation water) did not correspond to the initial concentration of mycotoxins in the maize grains before processing. Thus, further investigation should be carried out to elucidate the missing fraction.

7.3.2. Influence of the soybean powder processing method on *Fusarium* mycotoxins

The production of PSP was performed in triplicate using naturally contaminated soybean. The production process was carried out as described in Figure 7.2 using two processing methods (roasting and blanching). The results are shown in Table 7.3. As observed all the mycotoxins detected in the raw soybeans were lost after dehulling, except for FB_1 and ZEN, which were detected in the PSP at levels below the LOQ. The same trend was observed irrespective of the processing method.

Table 7.3. *Fusarium* mycotoxin concentration (µg/kg) in processing intermediates collected during soybean powder production with naturally contaminated soybean using two processing methods (roasting and blanching)

Processing method	Samples	Sample codes	Mycotoxins (µg/kg)				
			FB_1	FB_2	FB_3	ZEN	T-2
Roasting	Soybean	S1	52*	48*	39*	30*	23*
	After roasting	S6	34±12	13±18	<LOQ	26±14	<LOQ
	Processed soybean powder	S7	<LOQ	nd	nd	< LOQ	nd
	Roasted hull	S8	28±7	16±12	nd	17±9	nd
Blanching	Soybean	S1	52*	48*	39*	30*	23*
	After soaking	S2	46±17	23±2	nd	28±2	nd
	After blanching	S3	15±15	<LOD	nd	<LOQ	nd
	Processed soybean powder	S4	nd	nd	nd	nd	nd
	Blanched hull	S5	<LOQ	nd	nd	nd	<LOQ

FB_1, B_2, and B_3 = fumonisin B_1, B_2, and B_3; ZEN = zearalenone; T-2 = T-2 toxin; LOQ = limit of quantification; nd = not detected. Sample codes refer to sampling points as shown in Figure 7.2. Concentrations were calculated on dry weight basis. Experiment was performed in triplicate. *Concentration = there was no deviation in the values of the triplicate.

In order to ascertain the influence of processing on *Fusarium* mycotoxins, a highly-contaminated sample was needed, thus raw soybeans were spiked at two concentrations (1000 µg/kg and 500 µg/kg) of FB_1, DON, ZEN, and T-2. After spiking, the recovery was between 77% - 96% (Figure 7.5 and Table 7.4). A significant reduction of mycotoxins ranging from 19% to 76% was observed after roasting, irrespective of the mycotoxin or the initial concentration. This reduction agrees with the trend observed with the findings from PSP-processing with naturally contaminated soybeans, confirming the potential degradation of *Fusarium* mycotoxins. Roasting seems to affect FB_1 (91±25 µg/kg and 255±81 µg/kg) more, succeeded by DON (146±66 µg/kg and 439±33 µg/kg), and ZEN (193±52 µg/kg and 667±54 µg/kg), and then T-2 (218±11 µg/kg and 716±77 µg/kg) (Figures 7.5 and 7.6).

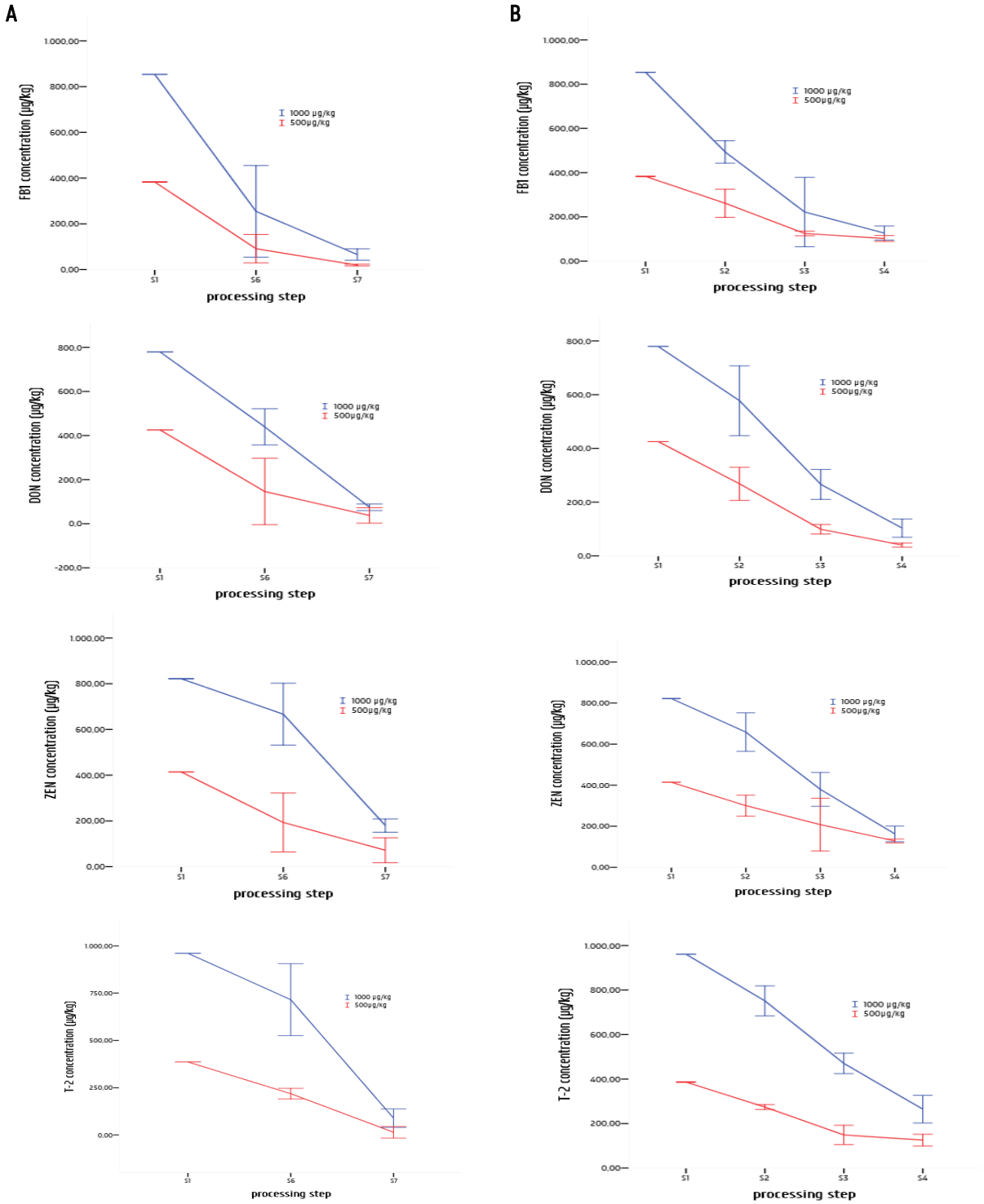

Figure 7.5. Concentrations (µg/kg) of fumonisin B_1 (FB_1), deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2) in the different processing steps of soybean powder (S1 = soybean, S2 = soaked bean, S3 = blanched bean, S4 = blanched soy powder, S6 = roasted soybean, S7 = roasted soybean powder). A = results from the roasting method, B = results from the blanching method. Experiments were performed in triplicate. Concentrations were calculated on dry weight basis.

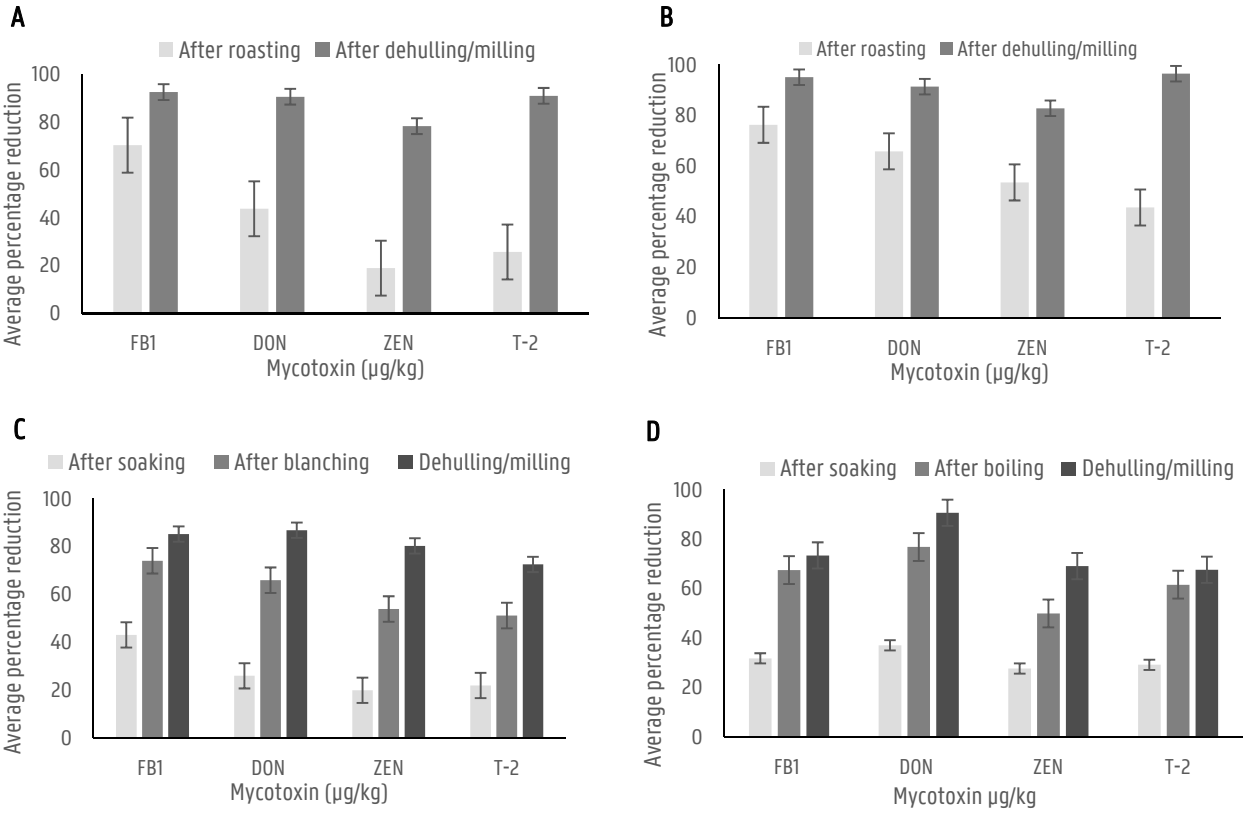

Figure 7.6. Average cumulative percentage reduction of regulated *Fusarium* mycotoxins (fumonisin B_1 (FB_1), deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2)) during processing of soybean powder with spiked soybeans using two processing methods. A: roasting method/soybean spiked at 1000 µg/kg; B: roasting method/soybean spiked at 500 µg/kg; C: blanching method/soybean spiked at 1000 µg/kg; D: blanching method/soybean spiked at 500 µg/kg. Experiments were performed in triplicate.

Earlier studies have reported a varying reduction of FB_1 during thermal processing which is majorly dependent on the temperature, type of heating, as well as the duration of the processing. A degradation of over 90% of the initial FB_1 concentration after heating at ≥175 °C for 60 min was recorded by Jackson et al. [44]. In another study, roasting of FB_1-artificially- and naturally- contaminated cornmeal samples at 218 °C for 15 min resulted in almost complete degradation of the toxin [45], which is higher than the reduction rate of 70% to 76% of FB_1 observed after roasting soybean at 180 °C for 8 min. This variation in results may be ascribed to the difference in roasting temperature and time, as well as the type of matrices. On the other hand, using the blanching processing method, a cumulative reduction of FB_1 between 67% to 74% after soaking/blanching was observed, while the influence of only blanching resulted in a 52% and 55% reduction. FB stability during thermal processing is often influenced by several

parameters, such as the amount of water in the medium containing FBs [46]. Jackson et al. [44] reported that FBs are more prone to destruction in dry environments compared to wet environments. This statement corroborated with the trend observed when the degradation rates of FB_1 in the two processing methods (roasting and blanching) were compared. In addition, $HYFB_1$ was detected after roasting and blanching at concentrations of 79±69 µg/kg and 16±14 µg/kg, respectively (Table 7.4). This was unexpected since hydrolysis of FB_1 to $HYFB_1$ is often caused by an alkaline treatment, although recent studies have revealed the possible contamination of raw cereals with this compound. However, Jackson et al. [47] observed a similar trend. In their study, they detected $HYFB_1$ representing <15% of the total FB_1 in an extruded corn product with or without the addition of glucose.

The reduction of DON and T-2 after roasting was observed at ranges between 44% - 66% and 25% - 43%, respectively. Whereas the cumulative effect of soaking and blanching led to a DON reduction of 66% - 77%, and T-2 by 51% -62% (Figure 7.6). Yumbe-Guevara et al. [48] reported the degradation of DON as a result of roasting and heating, however, they observed that DON degraded more during heating as compared to roasting, which correlates with the trend observed in this study. Similarly, degradation of *Fusarium* mycotoxins as a result of heat application (boiling) was also reported in Chapter 6. As observed in Chapter 6, the duration of heat application played an important role in the degradation of *Fusarium* mycotoxin. On the other hand, degradation of T-2 during heat treatment (baking and extrusion cooking) leading to significant levels of T-2 degradable compounds have been reported [50–52]. In the present study, HT-2 and NEO were detected after heat treatment (roasting and blanching), however, these compounds were detected only in the samples along the processing chain of highly-contaminated soybeans (spiked with 1,000 µg/kg) (Table 7.4). A similar trend was also observed with DON conjugates, with a reasonable amount of DON-3G (32±7.5 µg/kg) especially in blanching, while 3-ADON and 15-ADON were only traceable (<LOQ).

Table 7.4 Concentrations (µg/kg) of *Fusarium* mycotoxins detected in process intermediates and final products during processing of soybeans spiked with 1000 µg/kg using two different methods (roasting and blanching).

Processing method	Sample	Sample codes	*Fusarium* mycotoxin (µg/kg)												
			DON	FB_1	T-2	ZEN	$HYFB_1$	3-ADON	15-ADON	DON-3G	HT-2	NEO	α-ZEL	ß-ZEL	ZEN-14G
Roasting	Soybean	S1	780*	854*	962*	822*	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Roasted bean	S6	439±33	255±70	716±77	667±54	79±69	<LOQ	<LOQ	11±8.1	125±11	37±64	14±13	nd	39±15
	Roasted soybean powder	S7	75±6	66±10	89±21	180±12	15±17	nd	nd	<LOQ	67±10	nd	nd	nd	13±6
	Roasted hull	S8	413±36	497±42	451±26	553±24	133±116	<LOQ	nd	12±5	137±37	36±61	<LOQ	8±6	43±21
Blanching	Soybean	S1	780*	854*	962*	822*	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Soaked bean	S2	578±112	494±44	751±58	658±81	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Blanched bean	S3	266±48	222±136	470±40	379±71	16±14	nd	nd	32±7	24±5	nd	nd	nd	18±4
	Blanched soybean powder	S4	103±29	127±27	265±54	163±33	<LOQ	nd	nd	nd	nd	nd	nd	nd	<LOQ
	Blanched hull	S5	56±27	80±20	105±43	147±17	<LOQ	nd	nd	16±7	<LOQ	nd	nd	nd	<LOQ

DON = deoxynivalenol; FB_1 = fumonisin B_1; T-2 = T-2 toxin; ZEN = zearalenone; $HYFB_1$ = hydrolysed fumonisins; 3-ADON = 3-acetyl-deoxynivalenol; 15-ADON = 15-acetyl-deoxynivalenol; DON-3G = deoxynivalenol-3-glucoside; HT-2 = HT-2 toxin; NEO= neosolaniol; α-ZEL = α- zearalenol; ß-ZEL = ß-zearalenol; ZEN-14G = zearalenol-3- glucoside; <LOQ = less than limit of quantification; nd = not detected. Sample codes refer to sampling points as shown in Figure 7.2. Values are average of triplicates. Concentrations were calculated on dry weight basis. *Concentration = there was no deviation in the values of the triplicate.

As for ZEN, the concentration was reduced between 19% and 53% after roasting depending on the initial concentrations (Figures 7.5 and 7.6). After soaking/boiling, a cumulative reduction of ZEN ranged from 50% to 54%, with blanching contributing to a 31% - 42% reduction after soaking. ZEN is relatively heat stable, however in the presence of macro-components, ZEN may react to form conjugates [49]. The degradation observed in this study may be attributed to interaction between ZEN and matrix macro-components. This is in line with the study of Yumbe-Guevara et al. (2003), who reported a faster decomposition rate of ZEN in whole barley powder as compared to the ZEN reference standard. Bol et al. (2016) also reported degradation ranging from 75% - 95% of ZEN in different food products (cake, biscuit, bread, and pasta) as a result of thermal processing. Moreover, the glycoside of ZEN (ZEN-14G) was detected after roasting and blanching at a concentration of 39±15 µg/kg and 18±4.3 µg/kg, respectively, while α-ZEL was only detected in the roasted soybeans in low concentration. Evidence of α-ZEL occurring in processed soybean powder was revealed in Chapter 5. In addition, studies have reported a wide range of ZEN metabolites in processed food products [51].

As observed previously in Chapter 6, dehulling also plays an important role in detoxification, because of the accumulation of fungi and mycotoxins in the outer part, however, the extent of reduction is dependent on the fungal load and the extent of mycotoxin penetration into the grains [52,53]. Dehulling of soybeans in the two processing methods significantly reduced the concentrations of the mycotoxins irrespective of the initial concentration (Figures 7.5 and 7.6), thus, leaving the soybeans with relatively low mycotoxins levels (Table 7.4). However, soybeans processed with the blanching process were more contaminated irrespective of the mycotoxins, as compared to the hull. This observation suggests the possible penetration of these toxins into the soybeans during soaking and blanching. A similar trend was observed by Matumba [54], who reported the deposition of type B TH in maize endosperm after dehulling. The contamination of hull with mycotoxins as observed in this study, as well as in Chapter 6, represents a potential health risk to Nigerian livestock which are extensively fed with this by-product. The adverse impact of mycotoxins on animal production has been established [55,56]. Additionally, mycotoxins are carried over into animal food products, thus representing a potential route for human exposure to these toxins. A comparative analysis using Tukey's test between the two processing methods (roasting and blanching) of soybean powder across the different concentrations revealed no significant difference in reduction of ZEN, FB_1, DON, and T-2 with the processing initiated with raw soybeans spiked at higher concentration. While a significant difference ($p<0.05$) was observed for FB_1 and T-2 for processing performed with raw soybeans spiked at low concentration (500 µg/kg).

7.4. Conclusion

This study provides valuable information regarding the reduction of *Fusarium* mycotoxins during the traditional processing of *ogi* and processed soybean powder using naturally- and artificially- contaminated maize grains and soybeans. As observed in Chapter 6, there was a significant reduction of *Fusarium* mycotoxins along the different processing steps, irrespective of the processing method employed, the product type, mycotoxin type or the initial mycotoxin concentration. These factors contributed to the rate at which mycotoxins were reduced. Processing of *ogi* and PSP with naturally contaminated maize grains and soybeans, respectively, gave approximately 100% reduction for the tested mycotoxins, except for FB_1, $HYFB_1$, and FB_2 in *ogi* which levels were high in the raw maize. This implies that the concentration of mycotoxins in the final product is relative to the initial concentration before processing. This was also the trend when the spiked experiments were evaluated, suggesting that the use of good quality grains and beans for processing are important toward protecting consumers, especially infants and young children. In addition to the reduction of toxins observed in this study, an appreciable amount of some of the modified metabolites were detected. This is in line with literature on possible modification of free mycotoxins during processing [57]. The potential of modified mycotoxins to exert toxic effects has been established (Chapter 1), and as such should not be neglected. In spite of the observed reduction of mycotoxins along the process chain, it is noteworthy to mention that the detected mycotoxin proportions in the products and waste/hull does not correlate with the initial concentration. Unravelling the circumstance(s) of the undetected metabolites can be performed using HR-MS, and thus forms a potent foundation for further studies.

7.5. References

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GENERAL CONCLUSION

GENERAL CONCLUSION

This PhD study highlighted the status and knowledge gap as regards to *Fusarium* mycotoxin research in sub-Saharan Africa (SSA). Although, the problems caused by these mycotoxins in SSA are rarely documented, the limited studies conducted on the occurrence of the major *Fusarium* mycotoxins (fumonisins (FBs), trichothecenes (TH), and zearalenone (ZEN)) in food and food products in SSA revealed possibly high levels of these toxins. The absence of regulation is thus attributed to the lack of sufficient scientific data (occurrence, exposure, and toxicological data) and the socio-economic factors, such as public ignorance, (hidden) hunger, as well as political and economic instability. Based on the limited scientific information on the *Fusarium* mycotoxins in this region, and the recent concern with respect to the occurrence of emerging and modified *Fusarium* mycotoxins, this research was conceptualised as a Nigerian case study. Within this concept, the occurrence of a wide range of *Fusarium* mycotoxins and modified mycotoxins was investigated in major staple crops (maize, sorghum, millet, soybean, African locust bean, African mesquite bean, and African castor bean) and indigenous food products (*ogi,* soybean powder, *pito, burukutu, dawadawa*, *ogiri, okpehe, garri*, *lafun,* and *amala*) consumed in Nigeria. The analytical approach adopted for this investigation was the multi-analyte liquid chromatography-tandem mass spectrometry (LC-MS/MS) method validated for the different matrices.

The results clearly demonstrate the possible multi-occurrence of *Fusarium* and modified mycotoxins in Nigerian food products. The occurrence of *Fusarium* toxins in cereals and cereal-based fermented products (*ogi*) shows that FBs were the most dominating *Fusarium* mycotoxins with some of the samples exceeding the FB regulatory limits set by the European Union (EU). However, deoxynivalenol (DON) was the most frequent mycotoxin in popular traditional beers (*burukutu* and *pito*), while ZEN dominated in the processed soybean powder. Varying concentrations of *Fusarium* and modified mycotoxins were also detected in the traditional spices and their raw beans, as well as in *lafun, garri*, and *amala*. Although, *Fusarium* mycotoxins were detected in all the sample types, it is worth mentioning that cereals and cereal-based products were most contaminated with varying degrees of toxins. The co-occurrence of these mycotoxins presents a health risk due to the synergistic and/or additive effect, considering the fact that these food products are consumed almost on a daily basis. Hence, these toxins should not be neglected.

In addition, an enquiry into the effect of diverse traditional methods used for the production of these food products (*ogi*, processed soybean powder, *burukutu*, *pito, dawadawa, okpehe, ogiri*) on *Fusarium* mycotoxins (fumonisin B_1 (FB_1), DON, T-2 toxin (T-2), and ZEN) show that mycotoxin degradation is dependent on the process type, duration of processing, and the initial concentration. For all the food types, the concentration of these

toxins in the final products was significantly reduced irrespective of whether it was processed with naturally or artificially contaminated raw material. It is also important to highlight that some degradation products of FB_1, DON, T-2, and ZEN were detected in the process intermediates and final products. In spite of the observed reduction of mycotoxins along the process chain, it is noteworthy to mention that the detected mycotoxin proportions in the products and waste/hull did not correlate with the initial concentration. Unravelling the circumstance(s) of the undetected lost metabolites can be performed using high-resolution mass spectrometry (HR-MS). However, this was not within the scope of this PhD study. Further studies using HR-MS need to be performed along the processing chain of these products (including traditional spices, beers, and infant's foods) to evaluate the occurrence of a wide range of *Fusarium* mycotoxins. Relentless efforts need to be channelled towards rural and urban level advocacy on the need to use quality seeds of maize, millet or sorghum as raw materials for processing in different food products. Within this context, it is imperative and timely to educate the small-scale producers on the risk of mycotoxins and possible ways to reduce or avoid contamination. More effort should be channelled towards implementing good manufacturing and storage practices to prevent toxigenic fungi infestation and their associated mycotoxins. The results obtained necessitate the need for constant monitoring, as well as adoption of mitigation strategies towards the control of *Fusarium* mycotoxins in the Nigerian food system.

BROADER INTERNATIONAL CONTEXT, RELEVANCE, AND FUTURE PERSPECTIVES

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Broader international context

Agriculture remains the dominant economic activity in Africa. According to the World Bank report in 2010, the agricultural sector contributes about 12.7% in gross domestic product (GDP), and up to 60% of human employment [1]. In spite of abundant natural and human resource endowment in sub-Saharan Africa (SSA), poverty has remained pervasive, multifaceted and chronic. SSA does not produce enough food for the increasing human population, while remaining host to most undernourished countries. Given recent data available, it is estimated that approximately 39% of Nigerians live below the poverty line [2]. Nigeria was ranked 38th out of 76 on the 2014 Global Hunger Index [3], whilst another report indicated that the absolute number of food-insecure people was 17 million in 2012. This number is projected to rise to 43 million by 2022 if the situation is not addressed. In a region where food may not always be readily available, it is essential that consumed foodstuffs do not cause illness, as each foodborne disease outbreak results in a number of direct and indirect costs, in addition to the resultant human suffering.

It is important to highlight that due to SSA's food insecure status, food safety issues are rarely considered within the food value chain. Interestingly, the food industry is predominantly informal, consisting of small-open roadside stalls and mobile food vendors. Most of the people engaged in this booming food business do not observe, or are not aware of food hygiene and other best practices to ensure food safety and the environmental production facilities. Within this informal setting food safety laws are not feasible, and the sector provides service to millions of people who because of limited financial power depend on it.

The Codex Alimentarius Commission (CAC) was established in 1963 to develop international food standards, guidelines and recommendations to protect consumers' health, while ensuring fair practices in food trade. The political and cultural environment within SSA made CAC ineffective. A regional conference on food safety for Africa, jointly convened by Food and Agricultural Organisation (FAO) and World Health Organisation (WHO), took place in 2005, at the decision of the group of permanent representatives of the African region to FAO in Rome [4]. Participants from the 45 member countries from Africa recognized the need for increased national attention, and international, regional, and national cooperation to strengthen food safety systems in the countries of the region. The conference unanimously adopted a resolution recommending a nine-point five-year strategic plan for food safety in Africa for adoption by United Nations (UN) food and health agencies and the African Union, including numerous recommendations of practical actions to strengthen food safety systems in the region. The key elements of the plan included the following- food safety policies and programmes,

legislative and institutional aspects, standards and regulations, food inspection programmes and techniques, food analysis and food safety testing laboratories, monitoring food-borne diseases and the safety of foods on the market, participation in Codex, communication and stakeholder involvement (including industry officials and consumers), national, regional and international cooperation. Eleven years after this historic convention, unfortunately, much has not changed. Prevalent key challenges include lack of awareness of the socio-economic importance of food safety, paucity of data and information on incidence of food-borne disease outbreaks, lack of understanding of food safety and quality standards as outlined in international agreements, inability to enforce compliance with international standards and global best practices, inadequate infrastructure and resources to support scientific risk analysis and upgrading of food safety regulatory systems, inefficient supply chain and poor traceability systems.

In Nigeria, the food supply chain seems to have undergone considerable transformation as government intensifies its efforts to improve safety by reviewing and updating key components of the national food safety control system. Similarly, the agricultural and food industries are stepping up their food safety practices but the overall food safety situation is far from satisfactory. The Nation Agency for Food and Drug Administration and Control (NAFDAC) has a mandate to regulate and control the food industry in Nigeria. In addition, a revised National Policy on Food Safety and Implementation Strategy (NPFSIS) was inaugurated in 2014 under the supervision of Federal Ministry of Health in Nigeria. It is envisaged that this will engender the adoption of International best practices in the food sector, thereby enhancing consumer protection against fraudulent practices and supply of unwholesome and sub-standard foods. This policy will focus on building the capacity of both the public and private sectors, to strengthen the activities of the food safety control agencies, taking into account recent developments at national and international levels. Under this arrangement, the government will develop and implement mechanisms for training relevant stakeholders in the skills, tools and techniques for effective management of food safety at every point of the farm-to-table continuum. This effort was complimented in 2016 with the efforts of Partnership for Aflatoxin Control in Africa (PACA) and The Global Panel on Agriculture and Food Systems for Nutrition. Both bodies jointly issued a statement highlighting that food safety, and mycotoxins, are a significant threat not only to public health but also to agriculture and food systems in SSA.

The multiple regulatory agency system has been promoted in Nigeria with obvious historic drawbacks. It is difficult to ensure an integrated food control system because of the various historical, cultural and political perspectives in the Nigerian national development. National food control strategies should strive amidst challenges to identify, and assign roles to each agency to avoid duplication of efforts while enabling some

measures of coherence amongst them. For an integrated approach to be effective, a matching political will is needed towards effective collaboration and coordination between agencies across the farm-to-table continuum. Food security can be guaranteed when food is available, accessible, utilized, and stable [5]. A higher agricultural production is seen as a precondition to advance development and overcome cyclic poverty. Increased food availability translates into a reduction in prices for consumers thus improving overall access. Food safety according to WHO has to do with all measures taken to protect human health from harm, arising from the consumption of food when it is prepared and/or eaten according to its intended use. Food safety in SSA is subjugated to issues in the domain of food security, most especially in regions where food shortages are as a result of drought or political instability leading to civil unrest. It is increasing evident that factors related to food insecurity and malnutrition not only influence human health and welfare, but also affect social, economic, and political aspects of society. Countries within SSA can also increase their foreign earned income by exporting food products. However, these products must meet strict food safety and quality standards of the importing countries in order to gain access to the most lucrative markets. Studies have shown that the fixed costs of meeting standards tend to favour established exporters, and leads to a greater reduction in developing-country exports relative to those in developed countries [6].

Furthermore, practices aimed at improving food safety also reduce food losses. In order to effectively address food safety problems in SSA, food sufficiency needs to be attained. Under this scenario, prevalence and occurrence of mycotoxins in agricultural food products will continue to remain on the health and economic policy agenda of many countries. SSA is disadvantaged because of food limitation. It is imperative that proactive regulatory measures need to be taken to lower mycotoxin contamination. This action portends danger as it might lead to more food shortages and higher prices. This trend was observed in 1974 with the outbreak of aflatoxin hepatitis in Western India. "Starving to death today by not consuming contaminated food in order to live a better life tomorrow" is not a practical option. This trend is still relevant in SSA. Any intervention effort has to be pro-poor, well-focused, and cost-effective. A worrying dimension to this is that the general public's knowledge on food safety remains largely unaddressed by policy makers. Within the region, the food supply chain faces a number of challenges such as diversities in culture, lifestyles, and agricultural practices, mode of food transportation, handling, storage, preparation, transportation and eating habits. Most often, food contamination is not easily detected using traditional human senses. This leads to adverse health conditions upon consumption.

Numerous opportunities to improve food safety exist via technologies, value chain innovations and restructuring of food safety governance. These developments include: a reorientation of quality assurance protocols;

emphasis on the development of integrated and holistic food safety systems with a farm-to-fork approach; increased recognition of the respective roles of the different stakeholders along the food chain; increased food trade coupled with obligations under trade agreements; and advances in the control of foodborne hazards. Mutiga et al. [7] suggest that FBs, aflatoxin and other mycotoxin contamination are more widespread than previously articulated. They argued that investing on greater food safety surveillance will have more impact than focussing on post-harvest losses. Little has been done regarding mycotoxins in SSA because of the parallel attention to only *Aspergillus*-produced mycotoxin (aflatoxins). The climatic change scenarios have worsened the situation as recent studies reveal the exposure of SSA to so called temperate mycotoxin such as the *Fusarium* mycotoxins. It is therefore necessary to broaden the spectrum of mycotoxin monitored in food in this region. Investing on low-cost testing and analysis becomes essential [8]. Novel technologies can go a long way to address the challenges of food mycotoxin contamination. A baseline knowledge of pre- and post-harvest management strategies is a good starting point towards sustainable agricultural improvement. This will positively impact quality and nutritional value of foods, and natural resource conservation, while advancing local and international trade through competitiveness.

Relevance

Based on an FAO report, about 25% of the world's food crops have been estimated to be significantly contaminated with mycotoxins [9]. Losses accruing from mycotoxins as relate to human and animal health have many implications. Placinta et al. [10], argued that world-wide contamination of cereal grains and other feeds with *Fusarium* mycotoxins is a global problem. In developing countries, like Nigeria, tropical conditions like high temperatures and moisture, can lead to fungal proliferation and production of mycotoxins. Within the context of food safety, risk assessment should integrate food toxicology. Food toxicologists, while focusing on addressing food-related toxicological issues, should at the same time provide the appropriate knowledge in background to effectively support the evidence-based decision-making in food safety. A feasible drawback is the lack of understanding of the mechanisms and modes of action by foodborne mycotoxins including *Fusarium* species. The few countries in SSA with mycotoxin regulation have focus on aflatoxins, thus neglecting other mycotoxin especially *Fusarium* mycotoxin. Despite the evidence of occurrence of *Fusarium* mycotoxin in SSA and the possible link of *Fusarium* mycotoxin especially FBs to oesophageal cancer in this region, these toxins are still neglected. This translate to the limited occurrence data available thus leading to lack of regulatory guidelines on the control of *Fusarium* mycotoxins in the region. Gathering occurrence and toxicity data on *Fusarium* mycotoxins in staple food in SSA will serve as the basis for setting maximum level for these toxins in

agricultural commodities and processed food products. This PhD study was performed to proffer information on the possible occurrence of *Fusarium* mycotoxins and their modified forms in a variety of staple food commodities consumed in SSA using Nigeria as a case study.

The Nigerian food culture requires processing technologies that are appropriate and affordable to rural and urban economies. Processing techniques have been developed indigenously for diverse food products. This process offers various advantages including, improved food safety, improved nutritional values, enhanced flavour and acceptability, reduction in anti-nutrients, detoxification of toxigenic compounds, enhanced shelf-life and improved functional properties. Although, processing offers these benefits, the use of poor quality raw material often contaminated with fungi and mycotoxins overrule these advantages. In addition, processing of food is often done using rudimentary equipment under unhygienic environment, thus favouring the growth of micro-organisms such *Fusarium* species which may subsequently produce toxins. A missing gap in knowledge is the absence of base-line information as relates to *Fusarium* mycotoxin behaviour in the process chain. Such information can help optimize the process chain towards ensuring food safety. To this regard, the behaviour of *Fusarium* mycotoxins during processing of Nigerian traditional infant foods (*ogi* and processed soybean powder), traditional beers and spices were considered imperative and timely.

Food processing reduce mycotoxins levels in the food chain as observed in this study. The reduction is dependent on the initial concentration of the toxin, type of mycotoxin, and the processing method. Furthermore, it is important to mention the possible alteration of structural properties of mycotoxin during food processing. The exact effects and modification often remain unclear. Efficient reduction of mycotoxin exposure via food products requires the utilization of all available technologies from good agricultural practices (GAP), good manufacturing practices (GMP), hazard analysis and critical control point (HACCP), good storages practices (GSP) and selection of raw materials suitable for human consumption to the application of food processing technologies. There is limited evidence on effective, sustainable and scalable food safety interventions in SSA. Nigeria currently operates a multiple agency food safety control system which is mostly sectorial. Enactment and implementation of food safety legislation is also fragmented between three ties of government (federal, state, and local). Building on the existing food system platforms through research for development is likely to guarantee effective service delivery. This research seeks to enhance food security and improve livelihoods. Within this context, food safety challenges as relates to *Fusarium* mycotoxin contamination will be identified within the Nigerian food system/culture with a conscious intent to develop solutions through testing and deployment of mycotoxin management practices towards generating a sound knowledge-base on key biological,

environmental, institutional and policy drivers that influence contamination. This will result in enhanced local production, wealth creation, risk reduction, improve food quality, human health and livelihoods.

Future perspective

A practical consideration towards ensuring food safety is the adoption of GAP, GMP and HACCP, and GSP in SSA, especially Nigeria. Awareness and training should be given to farmers and stakeholders on the need for adoption of GAP. This approach presents a primary mechanism that will help reduce fungi and mycotoxin contamination of food products. Although climatic conditions cannot be controlled, agronomic practices can be modified to reduce the risk of fungal contamination. Postharvest control of mycotoxins can be addressed via GMP system to ensure that products meet food safety, quality, and legal requirements. Food manufacturers should have GMP in place with HACCP as complementary program. The HACCP plan in mycotoxin control provides a critical front line defence to prevent introduction of contaminants into the food and feed supplies. This established approach and other food quality assurance systems have been implemented by FAO, and should be promoted within developing countries like Nigeria.

A workable strategy would be the systematic development of centres of research expertise, and building research capacities aimed at establishing a database on health-related risks caused by mycotoxins. The need to grow the interest of the African scientific community towards increasing the research output in the region is imperative. To this end, building human resource capacity on mycotoxicology is a good starting point. National and regional hubs of excellence can be used as a platform. This will ensure a coordinated response approach, while postgraduate training using state of the art infrastructure will ensure sustainability. The present analytical methods in SSA may not provide accurate measurements of total contamination in food crops and products, and may underestimate the true levels. Lack of data precision and reproducibility is creating a significant bottleneck to progress. Recent advances in chromatographic techniques such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and high-resolution mass spectrometry (HR-MS) provide more choices and options to improve analytical performance. However, this is not the case in SSA as these instruments are rarely available, and when available, there is difficulty in maintaining and servicing due to epileptic power voltage, unskilled manpower for instrumentation maintenance, and absence of technical outlets of the manufacturing companies in the region. The establishment of technical offices within the national and regional hubs as proposed above will cushion these effects. In addition, developing the technical expertise of African nationals in respect to maintenance and management of these sensitive analytical instruments will be more pro-active, and in the long run more beneficial to SSA. In

midst of these challenges, the development of simple, precise, and low-cost diagnostic tests such as ELISA and LFIA can foster better mycotoxin monitoring in SSA. Governments within the region need to ensure a stability in policy, economics, and political environment to guarantee investment. An accelerated human capacity and infrastructural growth in mycotoxin research is proposed. The systemic institutional weakness of existing food regulatory agencies in SSA can be circumvented through the stakeholder's advocacy and regional partnerships. The establishment of a mycotoxin community of practice as well as strengthening of mycotoxicology scientific meetings represent a good starting point.

Another great challenge for the next decade is to mitigate the effect of climate change on crop production with a focus on sustaining crop and animal production levels with reduced fungal and mycotoxin contamination. A multi-pronged approach of using combined expertise will be critical in sustaining healthy food intake most especially in SSA. Management strategies need to put into perspective, the influence of input control measures of mycotoxigenic pathogens, the influence of environmental phenomenon, prevalence of non-symptomatic crops with toxin contamination, and the prevalence of quantitative resistance crops to both pathogen infections and toxin production. Furthermore, concerted efforts are required by farmers, post-harvest food specialists, breeders, agronomists, and technologists towards precise and strategic management systems in respect to the diverse staple food systems in the region.

The technical institutional and policy intervention measures are non-existent in most countries within SSA. Establishment of these frame works with legal back up will help in tackling problems that might arise in the context of screening the commodity value chain. In the author's opinion, there is no systemic surveillance of *Fusarium* mycotoxins diversity in toxigenic fungi in SSA. Such studies have been highly fragmentary. A regular surveillance survey in this regard will add value to already known knowledge, while bringing to fore a better understanding on the depth of problems inherent in SSA. An understanding of the evolutionary dynamics of these toxins is mostly needed. Breeding for field crop quantitative resistance is yet another option. Most often, field crop breeders are biased towards yield and disease resistance. An integrated team of postharvest specialists and mycotoxicologists should be part of the screening or phenotyping process of the breeder. Varietal releases should incorporate some sort of quantitative resistance to toxigenic fungi. Looking beyond the conventional breeding effort, genetic engineering can be exploited where specific genes of interest can be integrated to mitigate or prevent toxigenic progression of most fungi. Biological control measures using competitive exclusion principles in the various cropping systems can be exploited. This proved to be efficient in the control of pathogens [11] as exhibited by the use of atoxigenic strains of *Aspergillus flavus* to control aflatoxin-producing *A. flavus* [12].

The widespread concern over food safety in SSA and the growing evidence of the associated health burden and economic costs, makes it imperative that this region will receive greater attention in the future. Most worrisome is that food systems are evolving, giving rise to new food safety challenges. Assuring a safe food supply requires the highest level of political commitment to strengthen national systems. The establishment of country or regional hubs reference testing laboratories will go a long way in harmonising efforts within countries while promoting free flow of food products. Although proactive legal procedures on *Fusarium* mycotoxins control will certainly increase the burden of hunger with far reaching consequences, setting up *Fusarium* mycotoxins regulations in SSA would be a guiding pillar, principle, and requirement for food safety, and a mechanism to strengthen food control systems in the region (Broader international context). I subscribe to the words of Dr. Magaret Chan (former Director General of WHO) "*governments need to give food safety just as much attention as they devote to quality and safety of pharmaceutical products; not everyone needs to take medicine everyday but all people need food, each and every day*". She argues that the local food safety problem can rapidly become an international emergency. In her words "*investigation of an outbreak of foodborne disease is vastly more complicated when a single plate or package of food contains ingredients from multiple countries*." She suggests that efforts to prevent such emergencies can be strengthened, however, through development of robust food safety systems that drive collective government and public action to safeguard against chemical or microbial contamination of food. Food safety is a cross-cutting issue and shared responsibility that requires participation of non-public health sectors (i.e. agriculture, trade and commerce, environment, tourism), and support of major international and regional agencies and organizations active in the fields of food, emergency aid, and education.

It is essential to build truly sustainable human capacity in the biological sciences in developing countries, particularly in Africa. Active partnership between universities, national research programs, food processors, and farmers in Africa is very limited. With few good examples of functioning technology transfer systems, African scientists, particularly those just starting their careers, only see bits and pieces of the big picture, and are thus, not as effective as they could be. Capacity building is not just about training in new scientific tools and methods. Scientists, universities and research institutions in developing countries need many other skills and resources besides just technical know-how to effectively employ advanced science in their laboratories and ultimately develop products to help improve livelihoods. This philosophy lies at the heart of the MYTOX-SOUTH capacity building, which provides training courses in the research themes of toxigenic moulds, mycotoxins, and human health, and mycotoxin and animal health. MYTOX-SOUTH intends to harness the expertise and infrastructure available at Ghent University Belgium to strengthen the capacity of the Southern partners to tackle the mycotoxin problem and the associated food safety and food security issues. MYTOX-SOUTH is a partnership to improve food security & food safety through mitigation of mycotoxins at global level. This well-structured multi-

disciplinary partnership, which deals with all known aspects of mycotoxins and toxigenic mould issues, is able to provide the most adequate strategies and solutions for different stakeholders

The author subscribes to the lighthouse philosophy of building capacity, which contends that well-trained individual scientists or institutions in a developing country or region can serve as a beacon of best practices and skills to other individuals and institutions in the area to contribute to improved capacity throughout the region. The MYTOX-SOUTH aims to bring researchers into the network to serve as champions in research as related to food safety and mycotoxins. A critical approach to development is the engagement of African scientists early in their careers. Capacity building has to start at the undergraduate level, but it should extend to supporting post-doctoral scientists as well with the requirement to visit advanced laboratories to develop research and teaching skills. Training in SSA most often ends with the PhD because of limited funding opportunities. However, a post-doctoral position is also an avenue to develop more research and teaching skills to meet the evolving and dynamic challenges peculiar to SSA while presenting an opportunity to interact with great scientific minds.

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SUMMARY

SAMENVATTING

SUMMARY

Fusarium fungi are common plant pathogens causing several plant diseases. The presence of these moulds in plants exposes crops to toxic secondary metabolites called *Fusarium* mycotoxins. The most studied *Fusarium* mycotoxins include fumonisins (FBs), zearalenone (ZEN), and trichothecenes (TH). These arrays of toxins have been implicated as the causal agents of wide varieties of toxic health effects in humans and animals ranging from acute to chronic. Global surveillance of *Fusarium* mycotoxins has recorded significant progress in its control; however, little attention has been paid to *Fusarium* mycotoxins in sub-Saharan Africa, thus translating to limited occurrence data. In addition, legislative regulation is virtually non-existent. The emergence of modified *Fusarium* mycotoxins, which may contribute to additional toxic effects, worsens an already precarious situation. Chapter 1 of this study presents an overview on origin, chemistry, and distribution of *Fusarium* mycotoxins and their modified forms. It also highlights the status of *Fusarium* mycotoxins in sub-Saharan Africa, and the possible food processing mitigation strategies.

Nigeria as a country is culturally diverse with more than 250 distinct ethnic groups. The diverse ethnic background and numerous agro-ecologies have evolved specific food systems across regions. The country stands out as the most populated African country. These diversities define the rich cultural heritage of the Nigerian nation while prompting food and safety challenges including mycotoxins that are multifaceted. Consequently, a purposive research sampling technique that puts into consideration major agricultural producing areas and indigenous food systems was adopted in this study. The primary rationale is driven by the fundamental fact that the impact of *Fusarium* mycotoxins in Nigeria food systems is yet to be ascertained. The situation is made complex with the emergence of structurally modified secondary metabolites. In line with global health trends and food safety action policies of Nigeria, Chapter 2 defines contextually the study strategic intervention pathway and objectives towards examination of the status of *Fusarium* mycotoxins in Nigerian staple food while understanding the influence of different indigenous processing methods on the behaviour of *Fusarium* mycotoxins and their modified forms during production of traditional food products in Nigeria.

Maize, sorghum, and millet are very important cash crops. They are consumed on a daily basis in different processed forms in diverse cultural backgrounds. These crops are prone to fungi infestation, and subsequently may be contaminated with mycotoxins. A total of 462 samples comprising of maize (136), sorghum (110), millet (87), *ogi* (30), *burukutu* (54), and *pito* (45) were collected from randomly selected markets in four agro-ecological zones in Nigeria. Samples were assessed for *Fusarium* mycotoxins contamination using a multi-mycotoxin liquid chromatography-tandem mass spectrometry (LC-MS/MS) method in Chapter 3. Subsequently,

some selected samples were analysed for the occurrence of hidden fumonisins. Overall, 64% of the samples were contaminated with at least one toxin, at the rate of 77%, 44%, 59%, 97%, 78%, and 71% for maize, sorghum, millet, *ogi*, *burukutu*, and *pito*, respectively. FBs were the most dominant, especially in maize and *ogi*, occurring at the rate of 65% and 93% with mean values of 935 and 1128 µg/kg, respectively. The prevalence of diacetoxyscirpenol (DAS) was observed in maize (13%), sorghum (18%), and millet (29%), irrespective of the agro-ecological zone. Other mycotoxins detected were deoxynivalenol (DON), ZEN, and their metabolites, nivalenol (NIV), fusarenon-X (FUS-X), HT-2 toxin (HT-2), and hidden fumonisins. About 43% of the samples were contaminated with more than one toxin.

Subsequently, *Fusarium* mycotoxins were determined by a multi-mycotoxin LC-MS/MS method in cassava (*garri* and *lafun*) and yam (*amala*) products collected from some selected Nigerian markets (Chapter 4). Of the 94 samples analysed, 71% were contaminated with at least one mycotoxin at an incidence rate of 54% (*garri*), 72% (*lafun*), and 82% (*amala*). DON which ranged between 35 and 99 µg/kg was the most prevalent *Fusarium* mycotoxin in *garri* with an incidence rate of 38%, while the dominant mycotoxin in *lafun* (61%, range = 30-392 µg/kg) and *amala* (68%, range = 29 -155 µg/kg) was fumonisin B_2 (FB_2). Other mycotoxins detected from the food products included fumonisin B_1 (FB_1) and fumonisin B_3 (FB_3), ZEN, 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), deoxynivalenol-3-glucoside (DON-3G), T-2 toxin (T-2), DAS, and FUS-X.

Traditional spices and processed soybean powder play important roles in the diets of millions of Nigerians. Unfortunately, these products may be colonised by fungal infestation and subsequent production of mycotoxins. In Chapter 5 studies were done on a total of 192 food commodities consisting of 30 samples of raw soybean, 32 samples of processed soybean powder, 58 traditional spices (*dawadawa* = 17, *ogiri* = 20, *okpehe* = 21), and 72 indigenous beans (African locust bean (ALB) = 30, African castor bean (ACB) = 21, and African mesquite bean (AMB) = 21). These samples were analysed for *Fusarium* mycotoxins and their modified forms. All the sample types were found to be contaminated with *Fusarium* mycotoxins. In general, 76% (146/192) of the samples analysed were positive of at least one *Fusarium* toxin at an individual incidence rate of 47% (soybean), 100% (processed soybean powder), 74% (traditional spices), and 82% (indigenous beans). ZEN was the most prevalent mycotoxin in the processed soybean at an incidence rate of 81%, while FB_2 was the most dominant mycotoxin in traditional spices (33%) and indigenous beans (58%), respectively. Evidence of a cocktail of *Fusarium* mycotoxins was observed in all the sample types.

The findings in Chapters 3, 4, and 5 report for the first time a wide spectrum of *Fusarium* mycotoxins in Nigerian cereals and cereal-based products, indigenous beans and spices, soybean and processed soybean powder,

cassava and yam-based products. The results revealed that consumption of these food products may be a source of exposure to *Fusarium* mycotoxins, while causing potential synergistic health effects on the Nigerian population.

Chapter 6 describes the effect of processing methods used for the production of traditional beers (*pito* and *burukutu*) and spices (*dawadawa, ogiri*, and *okpehe*) on 4 regulated *Fusarium* mycotoxins using artificially contaminated raw materials. The results reveal a significant reduction of these toxins in all the final products irrespective of the toxin or the product. For the traditional spices, boiling played a significant ($p<0.05$) role in the reduction of the concentration of *Fusarium* mycotoxins irrespective of the product. The highest percentage reduction of DON (76±13% (SD)) was observed with AMB boiled for 12 h. Regarding ZEN, 74%, 53%, and 44% of reduction was recorded after boiling AMB, ALB, and ACB for 12 h, 4 h, and 3 h, respectively. This suggests an intrinsic relationship between boiling time and ZEN degradation. In addition, dehulling and fermentation further demonstrates a positive influence on the degradation of *Fusarium* mycotoxins. This trend was also observed during the production of traditional beers (*pito* and *burukutu)*, with the malting and brewing methods playing a major role in the degradation. In addition, some other metabolites including DON-3G, 15-ADON, α-zearalenol (α-ZEL), and β-zearalenol (β-ZEL) were detected in the final product.

The influence of processing methods used for the production of traditional infant food (*ogi* and processed soybean powder) on 4 regulated *Fusarium* mycotoxins using naturally and artificially contaminated raw materials in a laboratory-scale is reported in chapter 7. Generally, there was a significant reduction of all mycotoxins when compared to the initial concentration of the naturally contaminated raw maize during *ogi* processing. This trend was observed in the process chain initiated with artificially contaminated raw material. As observed in Chapter 6, this process based-reduction is dependent on several factors including, initial concentration of mycotoxins and process type. Reduction in concentrations of the mycotoxins started immediately after 36 h fermentation for all the mycotoxins (FB_1, DON, ZEN, and T-2) and proceeded along the process chain (milling, and dehulling). In addition, this study revealed the possible formation of glucosylated and acetylated DON (DON-3G and 3-ADON) at concentrations of 16±3.2 µg/kg and 9±5.5 µg/kg in the final *ogi* product. β-ZEL, hydrolysed fumonisin B_1 ($HYFB_1$), and HT-2 toxin (HT-2) were also detected at varying concentrations. In regard to soybean processing, a similar trend was observed with FB_1, ZEN, DON, and T-2 irrespective of the method used or the initial concentration. Other mycotoxins detected include 3-ADON, 15-ADON, DON-3G, HT-2, neosolaniol (NEO), α-ZEL, β-ZEL, and zearalenone-14-glucoside (ZEN-14G). Although, there were reductions in mycotoxin concentration as a result of processing, it is important to mention that an appreciable amount was detected in the final products, suggesting need for good quality raw materials.

SAMENVATTING

Fusarium schimmels zijn frequent voorkomende plant pathogenen die in staat zijn om een amalgaam aan plantenziekten te veroorzaken. De aanwezigheid van dergelijke schimmels in planten kan aanleiding geven tot de productie van toxische secundaire metabolieten, nl. *Fusarium* mycotoxinen. De meest grondig bestudeerde *Fusarium* mycotoxinen zijn fumonisinen (FBs), zearalenone (ZEN) en trichothecenen (TH). Deze toxinen worden oorzakelijk in verband gebracht met een wijde variëteit toxische -acute tot chronische- gezondheidsrisico's in zowel mens als dier. Het wereldwijd opvolgen en controleren van *Fusarium* mycotoxinen kent een significante progressie, doch wordter in sub-Sahara Afrika minimale aandacht besteed aan *Fusarium* mycotoxinen, wat aanleiding geeft tot het niet voorhanden zijn van data rond het voorkomen van deze toxinen. Alsook is wetgeving rond deze toxinen onbestaande in sub-Sahara Afrika. Het voorkomen van gemodificeerde *Fusarium* mycotoxinen, die potentieel aanleiding geven tot additionele toxische effecten, verergeren deze situatie. Het eerste hoofdstuk van deze doctoraatsthesis geeft de lezer een overzicht betreffende het voorkomen, de chemie en distributie van *Fusarium* mycotoxinen en de gemodificeerde vormen. Er wordt eveneens nadruk gelegd op de onderzoekstatus van *Fusarium* mycotoxinen in sub-Sahara Afrika en mogelijke voedselverwerkende mitigatie-strategieën.

Nigeria is een cultureel divers land met meer dan 250 ethnische groeperingen. Deze verschillende achtergronden en onnoemelijk veel agro-ecologieën geven aanleiding tot specifieke voedingssystemen over de gehele regio. Nigeria kent de grootste populatie van het Afrikaanse continent. De diversiteiten definiëren het rijke culturele erfgoed van de Nigeriaanse natie, doch impliceert vele uitdagingen inzake voedselveiligheid. Omwille van deze verscheidenheid werd een uitgekiend staalnameplan opgesteld waarbij de grootste agriculturele verwerkingsgebieden werden geïncludeerd en inheemse voedingssystemen werden bekeken. De primaire rationale van deze studie is het bestuderen van de impact van *Fusarium* mycotoxinen op Nigeriaanse voedingssystemen. De situatie wordt echter meer complex gemaakt door het voorkomen van gemodificeerde secundaire metabolieten. Hoofstuk 2 beschrijft contextueel de studie-strategie en de objectieven voor het examineren van *Fusarium* mycotoxinen in Nigeriaanse grondstoffen. Deze doctoraatsthesis werd behandeld in lijn met de globale gezondheidstrends en voedselveiligheid-actieplannen van Nigeria. Naast de analyse van de grondstoffen was het primordiaal de invloed van verschillende inheemse verwerkingstechnieken te bestuderen op het gedrag van *Fusarium* mycotoxinen en de gemodificeerde vormen gedurende de productie van traditionele voedingsproducten in Nigeria.

Mais, sorghum en gierst zijn de meest belangrijke gewassen in de Nigeriaanse economie. Ze worden op dagelijkse basis geconsumeerd in verschillende verwerkingsvormen met verschillende culturele bereidingstechnieken. Deze gewassen zijn gevoelig aan schimmel-infestatie waardoor het voorkomen van mycotoxinen een oorzakelijk verband is. Een totaal van 462 stalen waaronder mais (136), sorghum (110), gierst (87), *ogi* (30), *burukutu* (54) en *pito* (45) werden gecollecteerd vanuit random geselecteerde markten in vier agro-ecologische zones in Nigeria. De stalen werden onderworpen aan een analyse voor het bepalen van het *Fusarium* multi-mycotoxinen profiel met behulp van vloeistofchromatografie gekoppeld aan massaspectrometrie (LC-MS/MS, Hoofdstuk 3). Eveneens werden de geselecteerde stalen geanalyseerd op het voorkomen van *hidden* fumonisinen. 64% van de stalen waren gecontamineerd met minstens één mycotoxine met een incidentie van respectievelijk 77%, 44%, 59%, 97%, 78% en 71% voor mais, sorghum, gierst, *ogi*, *burukutu* en *pito*. FBs waren de meest voorkomende mycotoxinen, voornamelijk in mais en *ogi* met een incidentie van respectievelijk 65% en 93% met gemiddelde waarden van 935 en 1128 µg/kg. De prevalentie van diacetoxyscirpenol (DAS) in mais (13%), sorghum (18%) en gierst (29%) werd geobserveerd ongeacht de agro-ecologische zone. Andere gedetecteerde mycotoxinen waren deoxynivalenol (DON), ZEN en de metabolieten, nivalenol (NIV), fusarenon-x (FUS-X), HT-2 toxin (HT-2) en de *hidden* fumonisinen. 43% van de stalen waren gecontamineerd met meer dan één mycotoxine.

Hieropvolgend werden *Fusarium* mycotoxinen gedetermineerd met behulp van een multi-mycotoxinen LC-MS/MS methode in cassava- (*garri* en *lafun*) en yam-gebaseerde (*amala*) producten. Deze stalen werden gecollecteerd van verschillende geselecteerde Nigeriaanse markten (Hoofdstuk 4). Uitgaande van de 94 geanalyseerde stalen waren er 71% gecontamineerd met minstens 1 mycotoxine met een incidentie van 54% (*garri*), 72% (*lafun*) en 82% (*amala*). DON was het meest prevalente *Fusarium* mycotoxine met gehaltes die een range omvatten van 35 en 99 µg/kg in *garri* met een incidentie van 38%. Het meest voorkomende mycotoxine in *lafun* (61%, range = 30-392 µg/kg) en *amala* (68%, range = 29 -155 µg/kg) was FB_2. Andere gedetecteerde mycotoxinen in dergelijke voedingsproducten waren fumonisin B_1 (FB_1) en fumonisin B_3 (FB_3), ZEN, 3-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), deoxynivalenol-3-glucoside (DON-3G), T-2 toxin (T-2), DAS en FUS-X.

Traditionele specerijen en verwerkt sojaboon poeder maken een belangrijk deel uit van het dieet van miljoenen Nigerianen. Echter deze producten worden gekoloniseerd door schimmels met een plausibele mycotoxinen contaminatie tot gevolg. In Hoofdstuk 5 werden er in totaal 192 voedingsstalen geanalyseerd, meer bepaald 30 stalen ruwe sojabonen, 32 stalen verwerkt sojapoeder, 58 stalen traditionele specerijen (*dawadawa* = 17, *ogiri* = 20, *okpehe* = 21) en 72 stalen inheemse bonen (African locust bean (ALB) = 30, African castor bean (ACB) = 21,

and African mesquite bean (AMB) = 21). Deze stalen werden onderworpen aan analysen voor *Fusarium* mycotoxinen en de gemodificeerde vormen. Alle stalen toonden aan gecontamineerd te zijn met *Fusarium* mycotoxinen. In het algemeen werden 76% (146/192) van de stalen positief bevonden voor minstens één *Fusarium* mycotoxine met een individuele incidentie van 47% (sojaboon), 100% (verwerkt sojaboonpoeder), 74% (traditionele specerijen) en 82% (inheemse bonen). ZEN was het meest prevalente mycotoxine in de verwerkte sojabonen aan een incidentie van 81%, terwijl FB_2 het meest dominante mycotoxine was in traditionele specerijen (33%) en inheemse bonen (58%). Er werd aangetoond dat een cocktail van *Fusarium* mycootoxinen werd geobserveerd in alle staaltypes.

De bevonden resultaten in Hoofdstuk 3, 4 en 5 rapporteren voor de eerste maal het voorkomen van een wijd spectrum aan *Fusarium* mycotoxinen in Nigeriaanse granen en graanproducten, inheemse bonen en specerijen, sojaboon en verwerkt sojaboonpoeder, cassava en yam-gebaseerde producten. Het algemene resultaat toonde aan dat de consumptie van deze voedingsproducten een bron kunnen zijn van *Fusarium* mycotoxinen. Deze blootstelling kan hierdoor aanleiding geven tot potentiële synergistische gezondheidsrisico's op de Nigeriaanse populatie.

Hoofdstuk 6 beschrijft het effect van verschillende verwerkingsprocedures gebruikt voor het produceren van traditionele bieren (*pito* en *burukutu*) en specerijen (*dawadawa, ogiri* en *okpehe*) inzake 4 gereguleerde *Fusarium* mycotoxinen gebruikmakende van artificieel gecontamineerde ruwe grondstoffen. De bevonden resultaten tonen een significante reductie van de mycotoxinen in alle finale producten onafhankelijk van het type mycotoxine of het product. Voor de traditionele specerijen speelt het 'koken' een significante rol ($p<0.05$) in de reductie van *Fusarium* mycotoxinen. De meest significante reductie werd waargenomen voor DON (76±13% (SD)) na het koken met AMB gedurende 12 uur. Voor ZEN werd een reductie waargenomen van respectievelijk 74%, 53% en 44% na het koken met AMB, ALB en ACB gedurende 12 uur, 4 uur en 3 uur. Deze resultaten suggereren dat er een intrinsieke relatie is tussen de kook-duur en de ZEN-degradatie. Alsook concludeerde men dat het ontpellen en fermenteren een positieve invloed heeft op de degradatie van *Fusarium* mycotoxinen. Deze trend werd eveneens bevonden tijdens de productie van traditionele bieren (*pito* en *burukutu)* waarbij het mouten en brouwen een belangrijke rol speelden in de reductie. Alsook werden andere metabolieten in het finale product gedetecteerd, meer bepaald DON-3G, 15-ADON, α-zearalenol (α-ZEL) en β-zearalenol (β-ZEL).

In Hoofdstuk 7 wordt de invloed van verwerkingstechnieken op de productie van traditionele babyvoeding (*ogi* en verwerkt sojaboonpoeder) inzake 4 gereguleerde *Fusarium* mycotoxinen gebruikmakende van zowel natuurlijk als artificieel gecontamineerde ruwe grondstoffen op laboratoriumschaal beschreven. In het

algemeen was er een significante reductie van alle mycotoxinen, als men deze vergelijkt met de initiële concentratie van het natuurlijk gecontamineerde mais tijdens de *ogi* verwerking. Deze trend werd ook bemerkt in het proces met artificieel gecontamineerd materiaal. Zoals beschreven en geobserveerd in Hoofdstuk 6 kan deze reductie tijdens de verwerking afhankelijk zijn van verschillende factoren zoals de initiële concentratie van mycotoxinen en het verwerkingstype. De mycotoxinen reductie start direct na 36 uur fermentatie voor alle mycotoxinen (FB_1, DON, ZEN en T-2) en werd voortgezet over het gehele traject van de verwerkingsketen (vermalen en ontpellen). Alsook toonde deze studie de mogelijke formatie van geglucosyleerde en geacetyleerde DON (DON-3G en 3-ADON) aan met een concentratie van 16±3.2 µg/kg en 9±5.5 µg/kg in het finale *ogi* product. β-ZEL, hydrolysed fumonisin B_1 ($HYFB_1$) en HT-2 toxin (HT-2) werden eveneens gedetecteerd in variërende concentraties. Tijdens de sojaboon-verwerking werd een gelijkaardige trend geobserveerd voor FB_1, ZEN, DON, en T-2 onafhankelijk van de gebruikte methode of de initiële concentratie. Andere gedetecteerde mycotoxinen waren 3-ADON, 15-ADON, DON-3G, HT-2, NEO, α-ZEL, β-ZEL, en ZEN-14G. Het is belangrijk aan te halen dat een algemene reductie werd geobserveerd in de mycotoxinen concentratie als een resultaat van verwerking, doch dient men te melden dat steeds een aanzienlijk gehalte aan mycotoxinen in het finale product nablijft. Dit geeft aanleiding tot de noodzaak van het beschikken over een grondstof met optimale kwaliteit.

ANNEXES

Annex 1. Optimized ESI+ Mass spectrometric parameters

Component	Precursor ion (m/z)	Product ion (m/z)	Collision Energy (eV)	Cone Voltage (V)	Expected retention time (min)
FB_1	722.1	352.4* 704.4	36 29	56	9.19
FB_2	706.1	336.5* 688.5	40 29	61	11.8
FB_3	706.1	336.5* 688.5	37 31	54	10.31
DON	297.1	249.2* 231.2	10 15	26	4.11
3-ADON	339.0	231.2* 203.2	13 12	23	6.38
15-ADON	339.0	137.1* 321.2	10 10	26	6.40
DON-3G	476.1	248.6* 296.9	18 12	15	5.5
ZEN	319.1	187.2* 203.0	19 20	27	11.34
α- ZEL	321.3	285.4* 303.3	12 8	30	12.9
β-ZEL	321.0	285.1* 303.3	10 8	25	12.9
ZEN-14G	481.2	283.4* 319.2	15 15	37	11.3
NIV	313.1	125.0* 205.0	13 12	26	3.01
FUS-X	355.0	174.9* 137.0	20 25	18	5.12
T-2	489.1	245.1* 327.0	26 26	26	9.99
HT-2	447.1	345.3* 285.1	21 23	30	9.09
DAS	384.2	307.1* 247	12 14	21	7.84
NEO	400.0	305.3* 185.0	12 19	26	5.26
$HYFB_1$	406.5	388.5 370.5	13 11	23	8.45
$HYFB_2$	390.5	372.5 354.5	13 11	21	10.19
$HYFB_3$	390.5	372.5 354.5	15 15	20	9.5





Annex 2.1. Sampling memories

Raw cereal - sorghum

Steeping process

Germinated sorghum grains

Stirring the milled germinated grain in water (mashing process)

Boiling process

Fermentation process

Fermented *burukutu*

Burukutu in plastic cup

Annex 2.2. Pictorial flow diagram of *burukutu/pito* production described in Chapters 3 and 6

Raw cereals (maize or sorghum)

Steeping and fermentation process

Draining of the fermented grains

Cereal grains ready for wet milling

Sieved *ogi* (separation of starch - *ogi* from the hull using a fine cloth mesh)

Ogi packed in jute bag to reduced excess water added during sieving

Semi-dried *ogi*

Ogi ready for consumption

Annex 2.3. Pictorial flow diagram of *ogi* production described in Chapters 3 and 7

Raw material - soybeans

Roasting process

Roasted soybeans

Abrasion process using the mortar and pestle to aid separation of hull for the beans

Winnowing to remove the hull

Milling of soybeans

Soybean powder packed in plastic containers

Annex 2.4. Pictorial flow diagram of soybean powder production using roasting method described in Chapters 5 and 7

Annex 3 - Raw Data from the survey study for different food crops and food products

A.3.1. Maize, n=136, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	ZEN	α-ZEL	β-ZEL	ZEN-14G	HT-2	NIV	FUS-X	DAS	DON
2015/DSW1	73.62	238.59	56.85	-	-	-	18.66	-	-	-	-	147.01
2015/DSW2	111.43	506.18	79.62	-	-	-	-	-	-	-	-	-
2015/DSW3	-	76.2	-	-	-	-	-	-	-	-	-	-
2015/DSW4	135.21	362.26	86.49	-	-	-	-	-	-	-	-	-
2015/DSW5	70.25	359.99	153.92	-	-	-	23.8	-	-	-	-	48.21
2015/DSW6	43.96	-	-	-	-	-	-	-	-	-	-	-
2015/DSW7	45.49	-	-	-	-	-	16.5	-	-	-	-	89.25
2015/DSW8	-	-	-	-	-	-	-	-	-	-	-	-
2015/DSW9	-	-	-	-	-	-	-	-	-	-	-	-
2015/DSW10	38.74	-	-	-	-	-	-	-	-	-	-	-
2015/DSW11	-	-	-	-	-	-	-	-	-	-	-	-
2015/DSW12	-	-	-	-	-	-	-	-	-	-	-	-
2015/DSW13	44.4	-	39.24	-	-	-	21.2	-	-	-	2.19	66.1
2015/DSW14	-	101.12	-	-	-	-	-	-	-	-	-	-
2015/DSW15	-	202.65	-	-	-	-	-	-	-	153.6	2.26	-
2015/DSW16	-	-	-	-	-	-	-	-	-	-	-	-
2015/DSW17	-	-	21.84	-	-	-	-	-	-	-	-	69.4
2015/DSW18	65.59	-	69.2	-	-	-	23.02	-	270.5	-	-	-
2015/DSW19	-	-	-	-	-	-	-	-	-	-	2.29	74.12
2015/DSW20	105.86	113.81	61.97	-	-	-	-	-	-	-	-	-
2015/DSW21	131.99	254.34	116.31	-	-	-	17.5	-	-	-	-	-
2015/DSW22	365.63	1011.26	352.82	-	-	-	15.6	-	-	-	-	76.31
2015/DSW23	246.52	585.62	153.45	-	-	-	-	-	-	-	-	-
2015/DSW24	101.19	91.11	-	-	-	19.68	-	-	-	-	-	-
2015/DSW25	158.03	347.27	108.23	-	-	-	-	-	-	-	-	-
2015/DSY1	126.11	195.07	82.83	-	-	-	-	-	-	-	-	-
2015/DSY2	176.07	275.11	206.8	-	-	-	-	-	-	-	-	-
2015/DSY3	97.48	-	-	-	-	-	-	-	-	-	-	-
2015/DSY4	107.91	-	-	-	-	-	-	19.6	184.9	-	-	58.01
2015/DSY5	100.53	-	-	-	-	-	22.3	-	-	-	-	-
2015/SGW1	-	85.67	-	-	-	-	-	-	-	-	-	-
2015/SGW2	-	106.89	-	-	-	-	-	-	-	-	-	-
2015/SGW3	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW4	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW5	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW6	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW7	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW8	73.39	230.28	81.56	-	-	-	-	-	-	-	2.28	180.4
2015/SGW9	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW10	-	-	-	-	-	-	-	-	-	-	-	85.02
2015/SGW11	171.38	486.56	137.47	-	-	-	19.05	-	-	-	2.94	-
2015/SGW12	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW13	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW14	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW15	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW16	-	-	-	-	-	-	-	-	-	-	-	118.4
2015/SGW17	-	496.56	-	-	-	-	-	-	-	-	-	-
2015/SGW18	157.94	-	168.89	-	19.71	-	-	-	-	-	5.54	-
2015/SGW19	292.36	352.92	444.52	-	-	-	-	-	-	-	3.42	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, HT-2 = HT-2 toxin, NIV = nivalenol, FUS-X = fusarenon-X, DAS = diacetoxyscirpenol, DON = deoxynivalenol

A.3.1. Maize, n=136, results expressed in µg/kg (*Cont.*)

Sample code	FB_1	FB_2	FB_3	ZEN	α-ZEL	β-ZEL	ZEN-14G	HT-2	NIV	FUS-X	DAS	DON
2015/SGW20	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW21	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW22	-	315.46	-	-	-	-	-	-	-	-	-	-
2015/SGW23	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW24	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW25	-	-	-	-	-	-	-	-	-	-	-	58.16
2015/SGW26	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGW27	51.65	66.41	30.74	-	-	-	-	-	-	-	2.21	-
2015/SGW28	653.09	258.95	112.34	-	-	-	-	-	-	-	2.09	98.38
2015/SGY1	146.56	-	-	-	-	-	-	-	-	-	-	56.3
2015/SGY2	228.98	676.51	179.12	-	-	-	-	-	-	-	-	-
2015/SGY3	150.69	155.93	88.23	-	-	-	-	-	-	-	-	-
2015/SGY4	116.4	-	-	-	-	-	-	-	-	-	-	-
2015/SGY5	876.39	460.32	78.29	-	-	-	-	-	-	-	-	-
2015/SGY6	73.54	-	-	-	-	-	-	-	-	-	-	-
2015/SGY7	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGY8	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGW1	174.10	89.40	41.97	-	-	-	23.1	-	-	-	-	-
2015/NGW2	157.16	87.10	39.87	-	-	-	-	-	-	-	2.29	-
2015/NGW3	1781.59	1244.30	196.70	-	-	-	-	-	-	-	-	-
2015/NGW4	170.85	92.56	-	-	-	-	-	-	-	-	-	-
2015/NGW5	145.09	75.68	29.09	-	-	-	-	-	-	-	-	-
2015/NGW6	133.40	89.24	-	-	-	-	22.9	-	-	-	2.29	-
2015/NGW7	662.78	279.93	150.52	-	-	-	-	-	-	-	-	-
2015/NGW8	301.66	167.64	57.84	-	-	-	-	-	-	-	2.73	58.41
2015/NGW9	392.26	187.07	78.17	65	-	-	-	-	-	-	-	-
2015/NGW10	3245.48	2885.3	290.11	-	-	-	-	-	-	-	2.84	-
2015/NGW11	2871.44	2159.9	440.6	-	-	-	-	-	-	-	2.35	-
2015/NGW12	191.92	164.96	81.17	-	-	-	-	-	-	-	-	-
2015/NGW13	339.50	356.53	64.34	-	-	-	-	-	-	-	3.41	-
2015/NGW14	424.34	295.37	103.16	-	-	-	-	-	-	-	-	-
2015/NGW15	94.89	-	-	-	-	-	-	-	-	-	-	-
2015/NGW16	-	-	-	-	-	-	-	-	-	-	-	99
2015/NGW17	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGW18	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGW19	311.04	116.76	-	-	-	-	-	-	-	-	-	151
2015/NGW20	4788.99	2163.92	373.01	-	-	-	-	-	-	-	-	-
2015/NGW21	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGW22	487.49	-	95.27	-	-	-	-	-	-	-	-	-
2015/NGW23	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGW24	8222.27	-	-	-	-	-	-	-	-	-	-	-
2015/NGW1	234.51	-	-	-	-	-	-	-	-	-	-	-
2015/NGW2	572.61	424.21	52.04	-	-	-	-	-	-	-	-	-
2015/NGW3	1829.92	925.81	188.42	-	-	-	-	-	-	-	3.54	-
2015/NGW4	319.31	-	-	-	-	-	-	-	-	-	-	-
2015/NGW5	36.35	-	-	-	-	-	-	-	-	-	-	-
2015/NGW6	181.66	30.59	-	-	-	-	-	-	-	-	-	-
2015/NGW7	45.83	-	-	-	-	-	-	-	-	-	-	-
2015/NGW8	161.48	46.81	-	-	-	-	-	-	-	-	-	-
2015/NGW9	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGW10	-	-	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, HT-2 = HT-2 toxin, NIV = nivalenol, FUS-X = fusarenon-X, DAS = diacetoxyscirpenol, DON = deoxynivalenol

A.3.1. Maize, n=136, results expressed in µg/kg (Cont.)

Sample code	FB_1	FB_2	FB_3	ZEN	α-ZEL	β-ZEL	ZEN-14G	HT-2	NIV	FUS-X	DAS	DON
2015/NGW11	373.84	147.59	50.03	-	-	-	-	-	-	-	-	-
2015/NGW12	360.27	166.86	69.63	-	-	21.1	-	-	-	-	-	-
2015/NGW13	217.67	116.79	31.72	-	-	-	-	-	-	-	-	-
2015/NGW14	474.53	178.63	103.07	-	-	-	23.11	-	-	-	-	-
2015/NGW15	32.14	40.27	-	-	-	-	-	-	-	-	-	-
2015/NGW16	900.93	674.81	101.86	-	-	-	-	-	-	-	-	85
2015/SSW1	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSW2	150.43	-	-	-	-	-	-	-	-	-	7.6	-
2015/SSW3	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSW4	832.77	-	90.91	-	-	-	-	-	-	-	-	-
2015/SSW5	762.1	407.77	181.63	-	-	-	-	-	-	-	-	-
2015/SSW6	1257.93	984.27	124.62	-	-	-	-	-	-	-	-	220
2015/SSW7	841.19	410.57	72.4	-	-	-	-	-	-	-	-	-
2015/SSW8	127.3	190.64		-	-	-	-	-	-	-	-	-
2015/SSW9	321.74	238.42	34.11	-	-	-	-	-	-	-	-	-
2015/SSW10	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSW11	78.11	27.56	-	-	-	-	-	-	-	-	-	44
2015/SSW12	-	-	28.07	-	-	-	-	-	-	-	-	-
2015/SSW13	370.97	196.2	47.53	-	-	-	-	-	-	-	-	-
2015/SSW14	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSW15	636.38	355.27	76.86	-	-	-	-	-	-	-	-	69
2015/SSW16	229.06	146.74		-	-	-	-	-	-	-	-	-
2015/SSW17	879.68	496.83	114.4	-	-	-	-	-	-	-	-	225
2015/SSW18	2443.05	1107.05	198.62	-	-	-	-	-	-	-	-	-
2015/SSW19	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSW20	181.91	81.93	26.52	-	-	-	-	-	-	-	-	-
2015/SSW21	98.09	79.16	-	-	-	-	-	-	-	-	-	-
2015/SSW22	205.03	149.41	29.94	-	-	-	-	-	-	-	-	-
2015/SSW23	532.35	447.53	84.34	-	-	-	-	-	-	-	-	-
2015/SSY1	61.99	62.77	-	-	-	-	-	-	-	-	-	-
2015/SSY2	155.15	81.55	46.63	-	-	-	-	-	-	-	-	-
2015/SSY3	67.63	60.81	-	-	-	-	-	-	-	-	-	-
2015/SSY4	-	32.05	-	-	-	-	-	-	-	-	-	-
2015/SSY5	1266.31	572.13	212.94	-	-	-	-	-	-	-	-	-
2015/SSY6	59.38	-	-	-	-	-	-	-	-	-	-	-
2015/SSY7	57.1	55.96	-	-	-	-	-	-	163.2	-	7.54	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, ZEN-14G = zearalenone-14-glucoside, HT-2 = HT-2 toxin, NIV = nivalenol, FUS-X = fusarenon-X, DAS = diacetoxyscirpenol, DON = deoxynivalenol

A.3.2. Sorghum, n=110, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	DON	15-ADON	DON-3G	ZEN	α-ZOL	ß-ZOL	HT2	DAS	ZEN14G
2015/SGS01	-	-	-	-	-	-	-	-	-	-	3.91	-
2015/SGS02	-	-	-	-	-	-	-	-	-	-	3.88	-
2015/SGS03	-	-	-	-	-	-	-	-	21.39	-	3.08	-
2015/SGS04	-	-	-	-	-	-	-	-	-	-	3.77	-
2015/SGS05	-	-	-	-	-	8.67	-	-	-	-	2.25	-
2015/SGS06	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS07	-	-	-	-	-	9.61	-	-	-	30.63	-	-
2015/SGS08	-	-	-	-	-	9.12	-	-	-	-	-	-
2015/SGS09	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS010	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS011	-	-	-	-	-	11.63	-	-	-	-	-	20.2
2015/SGS012	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS013	69.8	-	-	-	-	12.73	-	-	-	-	-	-
2015/SGS014	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS015	-	-	-	-	-	16.02	37.75	-	-	-	-	-
2015/SGS016	-	-	-	-	33.47	11.12	-	-	-	30.97	-	-
2015/SGS017	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS018	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS019	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS020	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS021	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS022	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS023	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS024	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS025	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS026	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS027	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS028	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS029	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS030	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS031	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS032	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS033	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS034	-	-	-	119	-	15.03	-	-	-	-	-	-
2015/SGS035	-	-	-	-	-	-	-	-	-	-	-	-
2015/SGS036	-	-	-	-	-	-	-	-	-	11.35	-	-
2015/SGS037	-	-	-	-	-	-	-	-	-	-	4.67	-
2015/SGS038	70.6	-	-	-	-	-	-	-	-	11.5	13.03	-
2015/SGS039	-	-	-	-	-	-	-	-	-	11.28	-	-
2015/SGS040	-	-	-	-	-	-	-	-	-	-	8.53	-
2015/NGS01	-	-	-	-	44.16	16.74	-	-	-	30.81	-	22.3
2015/NGS02	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS03	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS04	-	-	-	-	-	15.64	-	-	-	-	-	-
2015/NGS05	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS06	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS07	-	-	-	-	-	14.72	-	-	-	30.6	-	-
2015/NGS08	-	-	-	-	-	14.37	-	-	-	-	-	-
2015/NGS09	-	-	-	-	-	16.2	-	-	-	-	-	-
2015/NGS010	-	-	-	-	-	16.53	-	-	-	-	-	-
2015/NGS011	-	-	-	92	-	14.61	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, ZEN-14G = zearalenone-14-glucoside

A.3.2. Sorghum, n=110, results expressed in µg/kg (Cont.)

Sample code	FB_1	FB_2	FB_3	DON	15-ADON	DON-3G	ZEN	α-ZOL	ß-ZOL	HT2	DAS	ZEN14G
2015/NGS012	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS013	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS014	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS015	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS016	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS017	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS018	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS019	45.3	-	-	-	-	13.54	-	-	-	-	-	-
2015/NGS020	-	-	-	-	-	55.31	-	-	-	-	-	-
2015/NGS021	67.3	-	-	-	-	33.61	-	-	-	-	2.02	15.1
2015/NGS022	76.1	40.5	30.97	-	-	43.17	-	32.54	-	-	-	-
2015/NGS023	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS024	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS025	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS026	-	-	-	-	-	-	-	32.3	-	-	-	-
2015/NGS027	-	-	-	89.18	-	-	-	-	-	-	-	-
2015/NGS028	45.6	-	-	-	-	25.11	-	-	-	-	-	-
2015/NGS029	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS030	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS031	-	-	-	-	-	55.35	-	-	-	-	-	-
2015/NGS032	-	-	-	-	-	33.11	-	-	-	-	-	-
2015/NGS033	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS034	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS035	-	-	-	-	-	-	-	-	-	-	-	-
2015/NGS036	-	-	-	-	-	44.64	-	32.8	-	-	4.97	-
2015/NGS037	-	-	-	-	-	63.36	-	-	-	-	3.76	-
2015/NGS038	-	-	-	-	-	-	-	-	-	-	3.46	-
2015/NGS039	-	-	-	-	-	-	-	-	-	11.41	-	-
2015/NGS040	-	-	-	-	-	-	-	-	-	-	3.1	-
2015/SSS01	-	-	-	-	-	21.50	-	-	-	-	-	-
2015/SSS02	60.97	-	-	-	-	-	-	-	-	-	-	-
2015/SSS03	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS04	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS05	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS06	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS07	78.4	55.10	45.98	-	-	-	-	-	-	-	2.99	-
2015/SSS08	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS09	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS010	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS011	-	-	-	-	-	-	-	-	-	-	2.09	-
2015/SSS012	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS013	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS014	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS015	-	-	-	-	-	-	-	-	-	-	2.62	-
2015/SSS016	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS017	-	-	-	-	-	-	-	-	-	11.37	-	-
2015/SSS018	-	-	-	-	-	-	-	-	-	-	4.18	-
2015/SSS019	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS020	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS021	60.9	-	-	-	-	-	-	-	-	-	16.25	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, ZEN-14G = zearalenone-14-glucoside

A.3.2. Sorghum, n=110, results expressed in µg/kg (Cont.)

Sample code	FB_1	FB_2	FB_3	DON	15-ADON	DON-3G	ZEN	α-ZOL	ß-ZOL	HT2	DAS	ZEN14G
2015/SSS022	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS023	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS024	-	-	-	-	-	-	-	-	-	-	4.01	-
2015/SSS025	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS026	-	-	-	-	-	-	-	-	-	-	4.35	-
2015/SSS027	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS028	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS029	-	-	-	-	-	-	-	-	-	-	-	-
2015/SSS030	-	-	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, ZEN-14G = zearalenone-14-glucoside

A.3.3. Millet, n=87, results expressed in µg/kg

Sample code	FB_1	FB_2	DON	15-ADON	ZEN	β-ZEL	HT-2	DAS	ZEN-14G
2015/10/SGMI1	92.03	64.87	-	-	1399.14	-	-	-	33.5
2015/10/SGMI2	-	-	-	-	68.46	-	-	-	-
2015/10/SGMI3	-	-	-	-	380.61	-	-	-	-
2015/10/SGMI4	-	-	-	-	-	-	-	-	-
2015/10/SGMI5	-	-	-	-	-	-	-	-	-
2015/10/SGMI6	41.93	47.01	-	-	422.05	-	-	-	-
2015/10/SGMI7	-	-	-	-	1250.72	-	-	-	-
2015/10/SGMI8	-	-	-	-	19.61	-	-	-	-
2015/10/SGMI9	-	-	-	-	301.79	-	-	-	-
2015/10/SGMI10	-	44.04	-	-	666.71	-	-	-	-
2015/10/SGMI11	-	-	81.01	-	-	-	-	-	-
2015/10/SGMI12	-	-	-	-	265.11	-	-	-	-
2015/10/SGMI13	80	-	-	-	-	-	-	11.94	-
2015/10/SGMI14	-	61.92	-	-	-	-	-	-	-
2015/10/SGMI15	-	58.34	-	-	-	-	-	-	-
2015/10/SGMI16	-	-	-	-	-	-	-	13.5	25.03
2015/10/SGMI17	-	-	-	-	-	-	-	-	-
2015/10/SGMI18	-	-	-	-	-	-	-	-	-
2015/10/SGMI19	-	77.69	199.48	-	-	-	-	24.79	-
2015/10/SGMI20	112.18	62.47	-	-	-	-	-	-	-
2015/10/SGMI21	-	-	-	-	-	-	-	-	-
2015/10/SGMI22	-	-	-	-	-	-	-	-	-
2015/10/SGMI23	18172.08	3892.00	-	-	-	-	-	-	-
2015/10/SGMI24	-	86.43	-	-	-	-	35.28	-	-
2015/10/SGMI25	-	99.24	-	-	33.82	-	-	4.21	-
2015/10/SGMI26	-	89.46	-	-	-	-	-	-	-
2015/10/SGMI27	-	-	-	-	-	-	35.33	3.66	-
2015/10/NGMI1	-	-	103.27	-	-	-	-	-	-
2015/10/NGMI2	-	-	-	-	-	-	-	-	-
2015/10/NGMI3	-	-	-	-	-	-	-	-	-
2015/10/NGMI4	83.9	52.66	-	-	198.01	-	-	3.74	19.6

FB_1, FB_2 = fumonisin B_1, B_2, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, ZEN = zearalenone, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, ZEN-14G = zearalenone-14-glucoside

A.3.3. Millet, n=87, results expressed in µg/kg (Cont.)

Sample code	FB_1	FB_2	DON	15-ADON	ZEN	β-ZEL	HT-2	DAS	ZEN-14G
2015/10/NGMI5	-	-	96.87	-	-	-	-	2.65	-
2015/10/NGMI6	-	-	-	-	-	-	-	-	-
2015/10/NGMI7	-	-	95.17	-	-	-	-	3.49	-
2015/10/NGMI8	-	-	-	-	-	-	-	-	-
2015/10/NGMI9	-	-	-	-	-	-	-	4.48	-
2015/10/NGMI10	-	-	-	-	-	-	-	-	-
2015/10/NGMI11	-	-	542.61	-	-	-	-	3.85	-
2015/10/NGMI12	-	-	-	-	-	-	-	-	-
2015/10/NGMI13	-	-	-	-	-	-	-	-	-
2015/10/NGMI14	-	-	-	-	-	-	-	3.99	-
2015/10/NGMI15	-	-	-	-	-	-	-	-	-
2015/10/NGMI16	-	-	-	-	-	-	-	-	-
2015/10/NGMI17	-	-	91.95	-	-	-	-	-	-
2015/10/NGMI18	-	-	-	-	-	-	-	-	-
2015/10/NGMI19	-	-	-	-	-	-	-	-	-
2015/10/NGMI20	-	-	93.58	-	-	-	-	2.77	-
2015/10/NGMI21	-	-	-	-	-	-	35.51	-	-
2015/10/NGMI22	-	-	-	-	-	-	-	-	-
2015/10/NGMI23	-	-	-	-	-	-	-	-	-
2015/10/NGMI24	43.32	103.00		-	-	-	36.18	-	-
2015/10/NGMI25	-	-	-	-	-	-	-	3.78	-
2015/10/NGMI26	-	-	-	-	-	39.28	-	4.36	-
2015/10/NGMI27	-	28.94	-	-	-	-	-	-	-
2015/10/NGMI28	35.12	38.61	-	-	20.05	-	-	-	-
2015/10/NGMI29	-	-	-	-	-	-	-	5.7	18.6
2015/10/NGMI30	-	-	-	-	-	-	-	3.42	-
2015/10/SSMI1	-	-	-	-	-	-	-	-	-
2015/10/SSMI2	-	-	-	-	-	-	-	-	-
2015/10/SSMI3	-	-	-	-	-	-	-	4.18	-
2015/10/SSMI4	-	-	86.85	-	-	-	-	-	-
2015/10/SSMI5	-	-	-	-	-	-	-	-	-
2015/10/SSMI6	-	-	-	-	-	-	-	-	-
2015/10/SSMI7	-	-	83.63	-	-	-	-	-	-
2015/10/SSMI8	-	-	-	-	-	-	-	-	-
2015/10/SSMI9	-	-	-	-	-	-	-	-	-
2015/10/SSMI10	-	46.51	183.63	11.33	-	-	-	-	-
2015/10/SSMI11	-	-	-	-	-	-	-	-	-
2015/10/SSMI12	-	41.3	-	-	-	-	-	-	-
2015/10/SSMI13	-	-	-	-	-	-	-	-	-
2015/10/SSMI14	-	-	-	-	-	-	-	-	-
2015/10/SSMI15	-	-	-	-	-	-	-	-	-
2015/10/SSMI16	-	-	-	-	-	-	-	-	-
2015/10/SSMI17	-	-	-	-	-	-	-	3.64	-
2015/10/SSMI18	-	-	-	-	-	-	-	-	-
2015/10/SSMI19	-	-	-	-	-	-	-	-	-
2015/10/SSMI20	-	-	-	-	-	-	-	3.23	-
2015/10/SSMI21	-	-	-	-	-	-	-	4.11	22.65
2015/10/SSMI22	-	-	-	-	-	-	-	3.44	-
2015/10/SSMI23	-	-	-	-	-	-	-	-	-
2015/10/SSMI24	-	-	-	-	-	-	-	-	-
2015/10/SSMI25	-	-	-	-	-	-	-	2.97	-

FB_1, FB_2 = fumonisin B_1, B_2, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, ZEN = zearalenone, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, ZEN-14G = zearalenone-14-glucoside

A.3.3. Millet, n=87, results expressed in µg/kg (Cont.)

Sample code	FB_1	FB_2	DON	15-ADON	ZEN	β-ZEL	HT-2	DAS	ZEN-14G
2015/10/SSMI26	-	-	-	-	-	-	-	3.25	-
2015/10/SSMI27	-	-	-	-	-	-	-	2.94	-
2015/10/SSMI28	-	-	-	-	-	-	-	-	-
2015/10/SSMI29	-	-	-	-	-	-	-	2.85	-
2015/10/SSMI30	-	-	-	-	-	-	-	-	-

FB_1, FB_2 = fumonisin B_1, B_2, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, ZEN = zearalenone, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol, ZEN-14G = zearalenone-14-glucoside

A.3.4. *Ogi*, n=30, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	15-ADON	ZEN	α-ZEL	ß-ZEL	HT-2	NIV	FUS-X	DON	DON-3G	ZEN-14G
2015/10.ogi/01	658	664.1	120.57	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/02	1427.8	1057.3	225.15	-	-	18.19	-	-	-	-	74	34	-
2015/10.ogi/03	622.68	563.32	-	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/04	978.94	893.68	-	-	-	-	-	-	159.6	-	-	-	-
2015/10.ogi/05	436.98	406.86	72.89	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/06	905.96	857.29	168.42	-	-	21.89	-	-	-	-	-	-	-
2015/10.ogi/07	266.69	182.55	50.74	60.2	-	-	18.17	-	-	-	-	-	-
2015/10.ogi/08	432.24	423.51	95.41	-	-	-	-	-	-	129.69	55	23	-
2015/10.ogi/09	758.15	596.51	141.47	-	-	-	-	-	135.6	-	-	-	-
2015/10.ogi/10	905.89	-	159.36	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/11	203.03	-	26.32	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/12	387.1	147.21	40.81	-	-	-	-	-	-	-	-	-	31.08
2015/10.ogi/13	70.11	55.06	-	-	-	-	-	-	-	136.98	49	18.6	-
2015/10.ogi/14	162.37	101.9	-	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/15	733.32	545.35	99.91	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/16	357.86	175.07	59.22	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/17	509.25	496.2	123.85	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/18	1902.5	1282.6	370.62	-	-	-	19.76	-	-	-	-	-	-
2015/10.ogi/19	379.57	285.96	79.19	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/20	326.92	279.12	76.56	-	39.21	-	19.61	-	-	-	-	-	-
2015/10.ogi/21	-	-	-	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/22	529.01	496.63	123.28	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/23	1468.5	1027.4	310.28	-	-	-	-	-	-	-	-	28.3	-
2015/10.ogi/24	-	-	-	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/25	136.77	133.04	26.57	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/26	66.75	46.72	33.05	-	-	-	-	-	-	-	66	44	-
2015/10.ogi/27	478.23	391.09	140.53	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/28	952.88	939.86	185.66	-	-	-	-	13.2	-	-	-	-	-
2015/10.ogi/29	109.2	40.23	-	-	-	-	-	-	-	-	-	-	-
2015/10.ogi/30	357.86	175.07	59.22	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, 15-ADON = 15-acetyl-deoxynivalenol, ZEN = zearalenone, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol, HT-2 = HT-2 toxin, NIV = nivalenol, FUS-X = fusarenon-X, DON = deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN-14G = zearalenone-14-glucoside

A.3.5. *Pito,* n=45, results expressed in µg/kg

Sample code	FB_1	FB_2	DON	15-ADON	3-ADON	DON-3G	ZEN	$HYFB_1$
2015/Pit.01	193.99	59.97	111.17	-	-	-	-	-
2015/Pit.02	-	-	73.33	-	-	-	-	-
2015/Pit.03	-	-	93.39	-	-	-	-	-
2015/Pit.04	-	-	82.11	-	-	-	-	-
2015/Pit.05	-	-	74.23	-	-	-	-	-
2015/Pit.06	104.46	-	-	-	-	-	-	-
2015/Pit.07	88.77	61.72	-	-	-	-	-	56.91
2015/Pit.08	-	-	-	-	-	-	-	95.64
2015/Pit.09	110.65	-	68.33	-	-	-	-	-
2015/Pit.10	-	-	97.25	-	-	-	-	-
2015/Pit.11	-	-	74.94	-	-	21.4	-	-
2015/Pit.12	-	-	-	-	-	28.2	-	-
2015/Pit.13	70.75	59.22	125.09	35.93	17.3	-	-	-
2015/Pit.14	-	-	-	-	-	-	-	-
2015/Pit.15	-	-	184.29	-	28.6	-	-	-
2015/Pit.16	-	-	-	-	-	-	-	-
2015/Pit.17	-	-	77.54	-	-	-	25.08	-
2015/Pit.18	-	-	-	-	37.2	-	-	-
2015/Pit.19	-	-	93.55	-	-	-	-	-
2015/Pit.20	66.78	43.59	-	-	-	-	22.85	-
2015/Pit.21	-	-	74.84	-	-	-	-	-
2015/Pit.22	-	-	-	-	-	-	-	-
2015/Pit.23	-	-	-	-	-	-	-	-
2015/Pit.24	-	-	91.15	-	-	-	-	-
2015/Pit.25	-	-	96.85	-	-	23.77	-	-
2015/Pit.26	-	-	-	-	-	-	-	-
2015/Pit.27	-	-	76.69	-	-	-	-	-
2015/Pit.28	-	-	116.76	-	-	18.17	-	-
2015/Pit.29	-	-	151.66	-	-	19.95	-	-
2015/Pit.30	-	-	102.76	-	-	-	-	92.54
2015/Pit.31	-	-	-	-	-	-	-	-
2015/Pit.32	-	-	-	-	-	-	-	-
2015/Pit.33	-	-	69.86	-	-	-	-	102.2
2015/Pit.34	-	-	125.62	-	-	-	-	56.91
2015/Pit.35	-	-	106.64	-	-	-	-	95.64
2015/Pit.36	-	-	-	-	-	-	-	-
2015/Pit.37	-	-	-	-	-	-	-	-
2015/Pit.38	-	-	-	-	-	-	-	-
2015/Pit.39	-	-	-	-	-	-	-	-
2015/Pit.40	124.16	58.15	180.63	-	-	-	-	-
2015/Pit.41	-	-	-	-	-	-	-	-
2015/Pit.42	154.76	-	-	-	-	-	-	-
2015/Pit.43	-	-	71.61	-	-	-	-	-
2015/Pit.44	-	-	64.66	-	-	-	-	-
2015/Pit.45	-	-	-	-	-	-	-	-

FB_1, FB_2 = fumonisin B_1, B_2, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, $HYFB_1$ = hydrolysed fumonisin B_1

A.3.6. *Burukutu,* n=54, results expressed in µg/kg

Sample code	FB_1	FB_2	HFB_1	DON	15-ADON	3-ADON	DON-3G	ZEN	ZEN-14G	α-ZEL	β-ZEL
2015/Bur.01	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.02	202.56	92.45	65.5	134.45	25.13	29.45	48.09	87.67	26.53	-	-
2015/Bur.03	-	-	-	61.44	24.29	-	-	-	-	-	-
2015/Bur.04	-	-	-	62.4	-	-	27.4	-	-	-	-
2015/Bur.05	100.77	62.41	-	69.39	-	-	-	-	-	-	-
2015/Bur.06	-	-	-	159.4	-	-	22.65	-	-	-	-
2015/Bur.07	-	-	-	241.6	-	-	-	-	-	-	-
2015/Bur.08	-	-	-	99.48	-	-	-	27.18	-	-	-
2015/Bur.09	233.66	172.73	99.2	124.4	-	-	-	-	-	-	-
2015/Bur.10	-	-	-	129.34	-	-	-	-	-	-	-
2015/Bur.11	-	-	-	152.43	-	-	-	-	-	-	-
2015/Bur.12	-	-	-	88.51	-	-	-	-	-	-	-
2015/Bur.13	-	-	-	127.37	-	-	-	-	-	-	-
2015/Bur.14	-	-	-	75.39	-	-	-	-	-	-	-
2015/Bur.15	-	-	-	71.34	-	-	-	-	-	-	-
2015/Bur.16	-	-	-	-	-	-	-	-	-	70.74	85.95
2015/Bur.17	-	-	-	-	-	-	-	-	-	32.15	107.31
2015/Bur.18	-	-	-	-	-	-	-	-	-	-	73.88
2015/Bur.19	316.34	202.74	152.3	-	-	-	51.5	-	-	-	-
2015/Bur.20	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.21	112.5	-	-	-	-	-	48.97	-	-	-	87.41
2015/Bur.22	-	-	-	145.41	-	-	-	-	-	-	-
2015/Bur.23	-	-	-	-	-	-	-	-	-	26.55	81.63
2015/Bur.24	-	-	-	79.4	-	-	-	-	-	35.66	-
2015/Bur.25	90.6	77.47	103.5	86.33	-	-	52.81	26.77	-	18.31	-
2015/Bur.26	-	-	-	85.45	-	-	-	-	-	-	-
2015/Bur.27	-	-	-	125.37	-	-	-	-	-	-	-
2015/Bur.28	-	-	-	192.44	-	-	-	-	-	-	-
2015/Bur.29	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.30	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.31	-	-	-	254.46	-	-	-	-	-	-	-
2015/Bur.32	-	-	-	69.32	-	-	-	-	-	-	-
2015/Bur.33	62.71	60.08	-	72.32	-	-	-	-	-	-	-
2015/Bur.34	80.12	70.11	73.2	81.42	-	-	-	-	-	-	-
2015/Bur.35	-	-	-	109.43	-	-	-	22.16	-	-	-
2015/Bur.36	70.42	-	-	185.59	-	-	-	-	-	-	-
2015/Bur.37	-	-	-	145.41	-	-	-	-	-	-	-
2015/Bur.38	168.78	-	77.6	99.35	-	-	23.54	-	-	-	-
2015/Bur.39	99.97	-	-	79.4	-	-	-	-	-	-	-
2015/Bur.40	-	-	-	86.33	-	-	-	-	-	-	-
2015/Bur.41	-	-	-	85.45	-	-	-	-	-	-	-
2015/Bur.42	118.6	-	-	125.37	-	-	-	-	-	-	-
2015/Bur.43	-	-	-	192.44	-	-	-	-	-	-	-
2015/Bur.44	-	-	-	225.47	-	-	25.62	-	-	-	-
2015/Bur.45	-	-	-	85.34	-	-	-	-	-	-	-
2015/Bur.46	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.47	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.48	-	-	-	-	-	-	22.45	-	-	-	-
2015/Bur.49	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.50	-	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2 = fumonisin B_1, B_2, $HYFB_1$ = hydrolysed fumonisin B_1, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, ZEN-14G = zearalenone-14-glucoside, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol

A.3.6. *Burukutu,* n=54, results expressed in µg/kg (Cont.)

Sample code	FB_1	FB_2	HFB_1	DON	15-ADON	3-ADON	DON-3G	ZEN	ZEN-14G	α-ZEL	β-ZEL
2015/Bur.51	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.52	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.53	-	-	-	-	-	-	-	-	-	-	-
2015/Bur.54	-	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2 = fumonisin B_1, B_2, $HYFB_1$ = hydrolysed fumonisin B_1, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, ZEN-14G = zearalenone-14-glucoside, α-ZEL = α-zearalenol, β-ZEL = β-zearalenol

A.3.7. Contamination of levels of fumonisins, total fumonisins, and modified fumonisinsnin maize, sorghum, millet, and *ogi*, results expressed in µg/kg

	Maize			Sorghum			Millet			*Ogi*		
Sample	FBs	Total FBs	Modified FBs	FBs	Total FBs	Modified FBs	FBs	Total FBs	Modified FBs	FBs	Total FBs	Modified FBs
1	696.0	3619.2	2923.2	69.8	349	279.2	156.9	238.5	81.6	1496.13	1795.4	299.2
2	2009.0	3214.4	1205.4	16.3	24.5	8.2	666.0	1838.2	1172.2	1065.25	1171.8	106.5
3	779.8	1013.7	233.9	21.3	78.8	57.5	840.0	1512.0	672.0	575.12	862.7	287.6
4	1290.5	3742.5	2452.0	147.6	428.0	280.4	88.9	364.7	275.7	264.27	269.6	5.3
5	3513.6	4567.7	1054.1	21.2	44.5	23.3	13.7	65.5	51.9	1378.58	1502.7	124.1
6	3.0	14.8	11.8	61.0	188.0	126.8	15.2	15.7	0.5	13.46	40.4	26.9
7	19.7	72.9	53.2	180.0	502.5	323.1	11.7	18.8	7.0	10.8	27.0	16.2
8	18.5	38.9	20.4	10.6	31.8	21.2	22.0	50.6	28.6	229.35	344.0	114.7
9	13.8	47.7	33.8	20.9	57.5	36.6	805.0	3059.0	2254.0	125.17	438.1	312.9
10	5.6	27.3	21.7	69.8	111.7	41.9	146.3	599.9	453.6	149.43	269.0	119.5

FBs = free fumonisins, total FBs = total fumonisins detected after hydrolysis, modified FBs concentration = the difference between the concentration of FBs and the concentration of total FBs after hydrolysis.

A.3.8. *Garri,* n=17, results expressed in µg/kg

Sample code	DON	DON-3G	FB_1	FB_2	ZEN	DAS	T-2
2015/Gar.01	-	-	-	-	-	-	-
2015/Gar.02	-	-	-	-	-	-	-
2015/Gar.03	-	-	-	-	-	-	-
2015/Gar.04	49.3	-	-	-	-	-	-
2015/Gar.05	98.7	11.5	-	-	-	-	-
2015/Gar.06	-	-	-	28.7	15.2	-	16.7
2015/Gar.07	45.2	20.3	65.5	-	-	-	-
2015/Gar.08	-	-	-	-	-	-	-
2015/Gar.09	-	-	-	-	-	-	-
2015/Gar.10	-	-	-	-	-	-	-
2015/Gar.11	70.2	-	48.1	32.4	-	-	-
2015/Gar.12	-	-	-	-	-	-	-
2015/Gar.13	-	-	-	-	-	-	-
2015/Gar.14	41.6	-	-	-	10.8	5.4	-
2015/Gar.15	-	14.7	58.6	39.7	-	-	-
2015/Gar.16	34.7	-	-	-	-	-	-
2015/Gar.17	-	-	79.6	65.3	-	9.6	19.4
2015/Gar.18	-	-	-	-	-	-	-
2015/Gar.19	66.5	-	45.1	35.1	16.5	-	-
2015/Gar.20	54.1	-	-	-	-	-	-
2015/Gar.21	-	-	60.2	-	13.1	-	21.9
2015/Gar.22	-	-	-	-	-	-	-
2015/Gar.23	-	-	-	-	-	-	-
2015/Gar.24	49.7	-	-	-	-	-	-

DON = deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, FB_1, FB_2 = fumonisin B_1, B_2, ZEN = zearalenone, DAS = diacetoxyscirpenol, T-2 = T-2 toxin

A.3.9. *Lafun,* n=36, results expressed in µg/kg

Sample code	DON	15-ADON	FB_1	FB_2	ZEN	DAS	FUS-X
2015/Laf.01	61	-	124.3	266.2	-	-	-
2015/Laf.02	-	-	-	211.4	-	-	-
2015/Laf.03	-	-	-	-	-	-	158.9
2015/Laf.04	85.6	-	45.2	89.2	-	6.7	-
2015/Laf.05	-	-	-	-	15.7	16.5	-
2015/Laf.06	-	-	-	-	-	-	-
2015/Laf.07	58.1	21.1	256.1	96.1	-	19.8	-
2015/Laf.08	-	-	-	-	-	-	-
2015/Laf.09	-	-	50.5	29.8	-	21.3	-
2015/Laf.10	90.8	35.5	-	109.1	13.3	22.1	-
2015/Laf.11	-	-	-	-	-	-	144.5
2015/Laf.12	-	-	212.6	129.5	-	-	-
2015/Laf.13	-	-	89.1	45.8	-	-	127.6
2015/Laf.14	-	-	47.3	45.2	-	-	-
2015/Laf.15	45.7	-	-	82.4	-	-	-
2015/Laf.16	-	-	-	87.1	-	-	-
2015/Laf.17	-	-	-	166.4	-	8.6	-
2015/Laf.18	56.8	32.6	65.5	89.5	-	11.2	-
2015/Laf.19	-	-	87.8	80.2	-	7.2	-
2015/Laf.20	-	-	241.8	391.8	-	12.4	-
2015/Laf.21	-	-	-	-	-	-	-
2015/Laf.22	-	-	-	-	-	-	-
2015/Laf.23	63.5	-	77.5	52.4	-	-	-
2015/Laf.24	-	-	-	-	-	-	-
2015/Laf.25	-	-	143.7	202.2	-	-	-
2015/Laf.26	-	-	-	-	-	-	-
2015/Laf.27	46.4	-	88.2	30.7	-	-	-
2015/Laf.28	-	-	47.1	29.5	-	-	-
2015/Laf.29	78.3	-	43.9	94.5	-	-	-
2015/Laf.30	-	-	-	-	-	-	-
2015/Laf.31	-	-	-	86.4	-	9.1	-
2015/Laf.32	-	-	-	-	-	-	-
2015/Laf.33	30.9	-	-	145.3	-	-	-
2015/Laf.34	-	-	-	-	-	-	-
2015/Laf.35	-	-	-	-	-	-	-
2015/Laf.36	-	-	138.4	-	-	15.5	-

DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, FB_1, FB_2 = fumonisin B_1, B_2, ZEN = zearalenone, DAS = diacetoxyscirpenol, FUS-X = fusarenon-X

A.3.10. *Amala,* n=34, results expressed in µg/kg

Sample code	DON	3-ADON	15-ADON	FB_1	FB_2	FB_3	ZEN	DAS	T-2	FUS-X
2015/Ama.01	-	-	-	-	-	-	-	-	-	-
2015/Ama.02	-	-	-	-	34.6	-	-	13.2	-	-
2015/Ama.03	56	35.2	14.2	35.5	76.2	-	-	-	-	-
2015/Ama.04	91.2	31	-	49.8	31.9	-	-	-	-	-
2015/Ama.05	-	-	-	-	29.1	-	-	21.4	-	-
2015/Ama.06	-	-	-	-	86.1	-	-	-	-	-
2015/Ama.07	-	-	-	-	-	-	-	-	-	-
2015/Ama.08	-	28.5	13.9	-	95	-	-	9.2	-	-
2015/Ama.09	39.1	-	-	46	-	-	-	-	-	-
2015/Ama.10	-	-	-	-	155	-	-	-	-	-
2015/Ama.11	-	-	-	-	-	-	-	3.4	-	-
2015/Ama.12	84.2	-	-	96.9	150	42		-	-	-
2015/Ama.13	-	-	-	-	-	-	-	-	-	-
2015/Ama.14	39.3	-	-	-	69.1	-	-	5.7	-	134.6
2015/Ama.15	-	-	-	-	-	-	-	-	-	-
2015/Ama.16	-	27.1	-	-	80.7	-	-	-	-	-
2015/Ama.17	-	-	-	-	-	-	-	-	-	-
2015/Ama.18	64.2	-	-	124.2	52.2	38.1	-	6.2	-	-
2015/Ama.19	80.5	-	-	-	144	-	-	-	-	-
2015/Ama.20	-	-	-	-	125	-	-	-	-	-
2015/Ama.21	104.3	-	-	-	64.4	-	-	-	-	-
2015/Ama.22	-	-	-	-	-	-	-	2.9	8.6	-
2015/Ama.23	-	-	-	-	-	-	-	-	-	-
2015/Ama.24	125.2	-	-	-	30.6	-	15.5	-	-	-
2015/Ama.25	-	-	-	-	-	-	-	-	-	-
2015/Ama.26	44	-	-	-	54.6	-	-	-	-	-
2015/Ama.27	49.2	-	-	-	55.2	-	-	-	-	-
2015/Ama.28	-	-	-	-	59.8	-	-	-	-	-
2015/Ama.29	-	-	-	-	-	-	9.4	3.2	-	-
2015/Ama.30	-	-	-	-	99.1	-	-	-	-	-
2015/Ama.31	57.7	-	-	56.4	55.6	-	-	-	-	-
2015/Ama.32	64.5	-	-	-	96.2	-	-	-	-	-
2015/Ama.33	-	-	-	-	76.7	-	-	-	-	-
2015/Ama.34	-	-	-	89.7	38.5	-	-	-	-	-
2015/Ama.35	-51.1	-	-	-	-	-	18.6	5.2	-	-

DON = deoxynivalenol, 3-ADON = 3-acetyl-deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, ZEN = zearalenone, DAS = diacetoxyscirpenol, T-2 = T-2 toxin, FUS-X = fusarenon-X

A.3.11. Soybean, n=30, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	ZEN	DAS	T-2	HT-2	NIV
2015/Soybea.01	-	-	-	-	-	-	-	-
2015/Soybea.02	-	-	-	-	5.3	-	-	-
2015/Soybea.03	-	-	-	-	-	-	-	-
2015/Soybea.04	-	-	-	21.8	-	-	-	-
2015/Soybea.05	53.28	-	-	19.2	-	-	-	-
2015/Soybea.06	52.31	48.29	39.31	-	-	-	-	-
2015/Soybea.07	-	-	-	-	-	-	-	-
2015/Soybea.08	-	-	-	-	-	-	-	-
2015/Soybea.09	-	-	-	-	-	-	-	-
2015/Soybea.10	-	-	-	12.5	-	-	-	-
2015/Soybea.11	-	-	-	-	-	-	-	-
2015/Soybea.12	-	-	-	-	-	-	-	-
2015/Soybea.13	-	-	-	-	-	-	-	-
2015/Soybea.14	-	-	-	-	-	-	-	-
2015/Soybea.15	-	-	-	-	-	-	-	-
2015/Soybea.16	-	-	-	22.04	2.1	-	-	-
2015/Soybea.17	-	56.8	-	-	-	-	20.7	-
2015/Soybea.18	-	-	-	-	-	-	-	-
2015/Soybea.19	-	-	-	-	-	-	21.94	-
2015/Soybea.20	-	-	-	7.52	3.5	-	-	-
2015/Soybea.21	-	66.45	40.1	26.07	-	-	-	-
2015/Soybea.22	-	-	-	-	-	-	-	-
2015/Soybea.23	-	-	-	-	-	-	-	-
2015/Soybea.24	-	-	-	21.75	2.9	23.1	19.75	-
2015/Soybea.25	-	-	-	25.08	-	-	-	-
2015/Soybea.26	-	-	-	-	3.7	21.31	15.61	-
2015/Soybea.27	-	-	-	-	-	-	-	-
2015/Soybea.28	-	-	-	-	-	-	-	-
2015/Soybea.29	-	-	-	29.82	-	-	20.18	-
2015/Soybea.30	-	-	-	-	-	-	25.02	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, ZEN = zearalenone, DAS = diacetoxyscirpenol, T-2 = T-2 toxin, HT-2 = HT-2 toxin, NIV = nivalenol

A.3.12. Processed soybean powder, n=32, results expressed in µg/kg

Sample code	DON	15-DON	DON-3G	FB_1	FB_2	FB_3	ZEN	α- ZEN	T-2	HT-2	FUS-X	NIV
2015/Soypow.01	-	-	-	-	-	-	34.7	-	-	-	-	-
2015/Soypow.02	-	-	-	34.19	-	-	29.56	-	-	-	-	-
2015/Soypow.03	-	-	-	-	-	-	54.5	-	-	-	-	-
2015/Soypow.04	-	-	-	143.25	78.58	39.35	42	-	-	-	-	-
2015/Soypow.05	-	-	-	269.75	181.18	48.75	64	-	-	-	66.22	69.8
2015/Soypow.06	-	-	-	-	-	-	53.4	-	-	-	-	82.6
2015/Soypow.07	-	84.83	-	-	-	-	31.1	-	-	-	-	-
2015/Soypow.08	-	84.72	-	-	-	-	37.6	-	-	-	-	103.8
2015/Soypow.09	85.02	88.83	-	-	-	-	37.5	-	-	-	-	-
2015/Soypow.10	-	-	-	-	-	-	33.43	-	-	-	-	-
2015/Soypow.11	-	99.11	-	-	-	-	-	-	-	-	-	78.96
2015/Soypow.12	-	-	-	-	-	-	59.4	-	13.22	-	-	-
2015/Soypow.13	-	-	-	-	-	-	-	-	10.1	22.2	-	94.11
2015/Soypow.14	-	-	-	-	-	-	29.6	-	-	24.81	-	-
2015/Soypow.15	-	-	-	-	-	-	-	-	10.9	-	-	73.93
2015/Soypow.16	-	110.18	-	-	-	-	27.2	-	-	-	-	-
2015/Soypow.17	-	-	12.25	-	-	-	28.39	-	-	31.58	275.95	-
2015/Soypow.18	-	-	-	-	-	-	33.4	-	-	-	98.39	-
2015/Soypow.19	-	107.47	-	-	-	-	38.1	-	-	-	-	-
2015/Soypow.20	61.14	112.54	12.62	90.96	51.04	43.53	92	-	-	-	-	-
2015/Soypow.21	-	-	-	98.62	64.21	44.79	58.3	-	-	-	-	-
2015/Soypow.22	-	-	-	-	-	-	388	-	-	-	-	-
2015/Soypow.23	-	-	13.74	-	-	-	29	21.96	-	26.4	93.24	-
2015/Soypow.24	69.39	-	13.57	99.13	44.34	43.14	-	21.77	-	-	-	76.18
2015/Soypow.25	111.63	-	13.54	118.76	112.15	55.86	-	20.29	-	-	158.35	-
2015/Soypow.26	-	-	-		-	-	67.3	-	-	-	-	-
2015/Soypow.27	-	-	-		-	-	-	-	-	31.6	-	-
2015/Soypow.28	-	97.77	-	86.23	-	-	28.5	-	-	33.0	-	-
2015/Soypow.29	-	71.12	-	-	-	-	97.4	-	-	-	-	-
2015/Soypow.30	-	95.22	12.93	-	-	-	32.85	-	-	35.41	87.41	-
2015/Soypow.31	180.16	-	14.21	-	-	-	79.8	-	-	-	-	-
2015/Soypow.32	-	-	-	104.8	-	-	57.65	19.19	-	25.1	86.39	-

DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, ZEN = zearalenone, α-ZEL = α-zearalenol, T-2 = T-2 toxin, HT-2 = HT-2 toxin, FUS-X = fusarenon-X, NIV = nivalenol

A.3.13. African locust bean, n=30, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	DON	15-ADON	ZEN	T-2	NIV	FUS-X	DAS
2015/lus.d01	-	242.01	-	-	10.1	83.08	-	130.83	151.36	13.85
2015/lus.d02	131.7	210.73	-	-	11.85	162.61	-	-	-	16.32
2015/lus.d03	136.12	841.14	-	-	-	181.3	-	-	-	-
2015/lus.d04	211.99	798.13	-	-	-	68.18	-	-	-	33.21
2015/lus.d05	86.82	196.64	-	-	-	73.99	-	89.54	-	-
2015/lus.d06	143.01	555.65	-	-	-	96.13	-	193.2	-	13.43
2015/lus.d07	-	-	-	-	-	-	-	-	-	-
2015/lus.d08	133.96	90.35	-	-	-	131.69	-	90.09	96.51	-
2015/lus.d09	95.32	142.48	-	-	-	141.9	-	100.34	-	-
2015/lus.d10	81.58	141.41	-	-	12.62	132.39	-	111.41	81.54	13.02
2015/lus.d11	-	-	-	-	-	-	-	-	-	-
2015/lus.d12	131.03	81.2	69.76	-	13.22	184.23	-	306.19	-	14.4
2015/lus.d13	213.03	96.09	78.6	93.75	-	95.82	9.96	-	101.81	12.74
2015/lus.d14	343.74	85.4	68.54	-	-	53.96	-	-	-	10.55
2015/lus.d15	-	54.64	48.76	-	-	163.68	-	-	-	-
2015/lus.d16	-	-	-	-	-	-	-	-	-	-
2015/lus.d17	89.78	84.73	49.17	-	-	145.51	-	-	-	-
2015/lus.d18	79.19	84.19	67.27	97.47	-	193.89	10.31	89.26	-	-
2015/lus.d19	84.48	74.42	76.86	-	-	94.08	-	67.77	-	9.72
2015/lus.d20	99.94	103.52	49.95	-	-	95.16	-	-	75.78	-
2015/lus.d21	-	- -	-	-	-	-	-	-	-	-
2015/lus.d22	130.26	164.85	-	-	-	64.79	-	-	-	10.05
2015/lus.d23	-	-	-	-	-	-	-	-	-	-
2015/lus.d24	-	-	-	-	-	-	-	-	-	-
2015/lus.d25	99.97	142.7	-	-	-	73.7	-	84.58	121.47	-
2015/lus.d26	-	-	-	-	-	-	-	-	-	-
2015/lus.d27	-	-	-	-	-	-	-	-	-	-
2015/lus.d28	90.49	54.07	-	-	-	163.45	-	91.55	-	-
2015/lus.d29	110.46	554.95	-	-	-	84.15	-	-	-	-
2015/lus.d30	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, ZEN = zearalenone, T-2 = T-2 toxin, NIV = nivalenol, FUS-X = fusarenon-X, DAS = diacetoxyscirpenol

A.3.14. African castor bean, n=21, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	DON	ZEN	α-ZEL	T-2	HT-2	NIV	DAS
2015/Cas.o01	126.48	169.81	80.94	-	56.64	-	-	-	-	-
2015/Cas.o02	187.86	65.53	-	-	87.29	-	-	-	-	-
2015/Cas.o03	127.5	91.39	-	-	-	19.54	-	52.26	-	-
2015/Cas.o04	211.32	72.65	-	-	-	-	-	38.88	-	-
2015/Cas.o05	-	88.89	-	-	-	23.37	-	-	-	-
2015/Cas.o06	150.53	102.79	61.92	-	86.67	-	-	-	105.85	-
2015/Cas.o07	-	68.97	-	-	-	-	-	56.94	-	-
2015/Cas.o08	-	-	-	-	58.56	19.88	-	-	-	-
2015/Cas.o09	-	96.97	-	158.41	57.16	-	-	81.99	-	-
2015/Cas.o10	-	-	-	-	76.58	-	-	-	-	-
2015/Cas.o11	90.62	86.71	72.58	108.12	-	-	-	-	-	-
2015/Cas.o12	127.12	78.41	58.3	-	-	-	105.23	-	-	6.28
2015/Cas.o13	-	-	-	-	-	-	-	-	94.77	-
2015/Cas.o14	-	-	-	227.53	84.64	-	125.38	-	-	-
2015/Cas.o15	-	-	-	-	-	-	-	-	-	-
2015/Cas.o16	-	-	-	-	-	-	-	47.91	-	-
2015/Cas.o17	-	-	-	-	-	-	-	-	-	-
2015/Cas.o18	157.55	92.5	-	-	-	-	135.39	49.54	-	-
2015/Cas.o19	98.83	87.89	80.13	-	94.7	-	-	-	-	5.18
2015/Cas.o20	87.29	172.7	70.89	-	89.63	-	-	69.22	-	-
2015/Cas.o21	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, DON = deoxynivalenol, ZEN = zearalenone, α-ZEL = α-zearalenol, T-2 = T-2 toxin, HT-2 = HT-2 toxin, NIV = nivalenol, DAS = diacetoxyscirpenol

A.3.15. African mesquite bean, n=21, results expressed in µg/kg

Sample code	FB_1	FB_2	DON	15-ADON	DON-3G	ZEN	T-2	HT-2	FUS-X	DAS
2015/Mes.o01	-	97.84	-	-	6.24	86.6	-	-	110.83	8.32
2015/Mes.o02	-	84.97	-	29.27	-	57.11	-	-	106.83	7.39
2015/Mes.o03	172.59	93.29	-	-	-	47.37	-	-	98.11	8.21
2015/Mes.o04	150.09	-	-	49.37	8.26	115.92	-	120.73	112.39	-
2015/Mes.o05	-	89.6	-	48.19	-	107.75	-	-	-	8.62
2015/Mes.o06	98.09	93.6	-	15.35	9.06	-	-	97.69	-	6.93
2015/Mes.o07	-	114.59	-	-	9.23	34.83	-	110.31	-	6.2
2015/Mes.o08	-	-	156.65	-	-	58.76	-	98.77	-	5.03
2015/Mes.o09	-	-	218.44	-	6.36	20.56	66.82	90.13	134.45	7.15
2015/Mes.o10	-	-	-	-	-	-	-	89.32	-	8.33
2015/Mes.o11	158.45	-	82.11	-	35.76	58.86	-	-	-	-
2015/Mes.o12	108.48	-	-	-	20.44	57.91	-	-	-	2.3
2015/Mes.o13	-	-	205.93	-	19.82	-	-	-	-	6.68
2015/Mes.o14	-	-	287.56	-	9.78	-	-	-	131.76	-
2015/Mes.o15	-	-	-	27.43	12.29	67.87	-	-	-	-
2015/Mes.o16	-	-	-	58.44	-	-	-	-	123.34	-
2015/Mes.o17	-	-	-	-	-	65.57	-	88.26	142.42	-
2015/Mes.o18	192.77	165.54	-	19.85	-	-	-	-	-	6.72
2015/Mes.o19	-	-	-	-	-	47.4	-	-	-	2.19
2015/Mes.o20	-	88.24	-	-	-	45.86	-	-	-	5.09
2015/Mes.o21	-	-	-	-	-	-	-	-	-	-

FB_1, FB_2 = fumonisin B_1, B_2, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, DON-3G = deoxynivalenol-3-glucoside, ZEN = zearalenone, T-2 = T-2 toxin, HT-2 = HT-2 toxin, FUS-X = fusarenon-X, DAS = diacetoxyscirpenol

A.3.16. *Ogiri,* n=20, results expressed in µg/kg

Sample code	FB_2	DON	15-ADON	ZEN	HT-2	NIV	DAS
2015/giri.01	141.56	-	-	-	80.5	-	-
2015/giri.02	95.54	-	30.03	-	-	-	-
2015/giri.03	-	-	-	82.7	-	284.5	6.09
2015/giri.04	-	-	-	-	-	-	-
2015/giri.05	-	105.95	-	75.33	-	-	-
2015/giri.06	-	-	20.15	-	95.05	357.62	-
2015/giri.07	-	-	28.31	-	-	-	-
2015/giri.08	-	155.13	-	-	-	-	-
2015/giri.09	-	89.85	24.84	-	-	-	-
2015/giri.10	126.44	-	-	-	-	126.36	-
2015/giri.11	151.37	-	-	-	-	-	-
2015/giri.12	-	-	-	-	-	-	-
2015/giri.13	-	-	-	-	-	-	4.79
2015/giri.14	-	-	-	-	-	-	-
2015/giri.15	-	-	-	-	-	-	-
2015/giri.16	-	-	-	73.28	-	-	-
2015/giri.17	-	-	-	53.34	-	-	-
2015/giri.18	-	-	-	-	-	-	-
2015/giri.19	-	-	-	-	-	-	-
2015/giri.20	-	-	-	-	-	-	-

FB_2 = fumonisin B_2, DON = deoxynivalenol, 15-ADON = 15-acetyl-deoxynivalenol, ZEN = zearalenone, HT-2 = HT-2 toxin, NIV = nivalenol, DAS = diacetoxyscirpenol

A.3.17. *Okpehe,* n=20, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	DON	ZEN	T-2	DAS
2015/0kp.01	162.23	111.5	-	-	-	-	32.09
2015/0kp.02	126.58	142.41	-	-	-	-	28.79
2015/0kp.03	182.44	-	-	-	112.73	-	16.27
2015/0kp.04	-	-	-	118.02	-	-	-
2015/0kp.05	94.06	95.91	-	-	115.37	-	-
2015/0kp.06	109.59	87.37	76.56	-	87.63	-	-
2015/0kp.07	99.39	-	51.41	-	-	52.21	-
2015/0kp.08	148.26	78.59	-	-	-	-	-
2015/0kp.09	-	-	-	89.85	-	-	12.69
2015/0kp.10	-	-	-	-	77.68	-	10.14
2015/0kp.11	-	-	-	-	-	-	-
2015/0kp.12	-	-	-	-	-	-	-
2015/0kp.13	-	-	-	163.96	-	-	-
2015/0kp.14	-	-	-	116.09	-	-	-
2015/0kp.15	-	-	-	149.1	-	-	-
2015/0kp.16	-	-	-	96.18	-	-	-
2015/0kp.17	-	-	-	142.84	-	-	-
2015/0kp.18	-	-	-	-	-	-	-
2015/0kp.19	-	-	-	-	-	-	-
2015/0kp.20	-	-	-	-	-	-	-
2015/0kp.21	-	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, DON = deoxynivalenol, ZEN = zearalenone, T-2 = T-2 toxin, DAS = diacetoxyscirpenol

A.3.18. *Dawadawa,* n=17, results expressed in µg/kg

Sample code	FB_1	FB_2	FB_3	15-ADON	ZEN	T-2	HT-2	DAS
2015/Daw.01	116.68	170.05	-	-	-	31.31	-	-
2015/Daw.02	105.08	-	-	-	-	-	-	3.14
2015/Daw.03	85.37	51.12	-	-	-	-	-	5.14
2015/Daw.04	-	157.88	-	-	-	21.41	-	6.15
2015/Daw.05	78.1	58.81	-	-	-	-	-	-
2015/Daw.06	-	156	-	-	-	-	-	-
2015/Daw.07	-	-	-	-	-	32.13	-	-
2015/Daw.08	-	54.78	-	-	85.83	29.28	-	-
2015/Daw.09	-	71.04	92.48	28.68	-	25.25	-	-
2015/Daw.10	164.62	53.78	-	22.61	56.96	24.94	-	-
2015/Daw.11	-	49.87	-	-	-	-	-	-
2015/Daw.12	-	-	-	-	33.49	-	-	-
2015/Daw.13	85.22	-	131	-	33.23	-	-	-
2015/Daw.14	93.68	42.84	35.99	15.62	77.34	-	-	-
2015/Daw.15	72.34	-	-	16.6	41.09	-	58.18	-
2015/Daw.16	-	-	-	-	-	-	-	-
2015/Daw.17	-	-	-	-	-	-	-	-

FB_1, FB_2, FB_3 = fumonisin B_1, B_2, B_3, 15-ADON = 15-acetyl-deoxynivalenol, ZEN = zearalenone, T-2 = T-2 toxin, HT-2 = HT-2 toxin, DAS = diacetoxyscirpenol

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Education

2015 – 2017	PhD in Bio-analysis, Ghent University, Ghent, Belgium. Dissertation: *Fusarium* mycotoxins and their modified forms in Nigerian foods: occurrence and influence of traditional processing methods.
2010 – 2012	MTech in Food Technology, University of Johannesburg, Johannesburg, South Africa. Dissertation: A survey of South African commercial feed grade maize for mycotoxins with particular reference to fumonisins using different analytical techniques.
2001 – 2005	BSc (Hons) in Food Science and Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. Dissertation: Evaluation of the efficiency of yeast isolates from palm wine in fruit wine production.

Work experience

2014- present	Academic staff in Department of Food Science and Technology, Michael Okpara University of Agriculture Umudike, Abia State Nigeria. *Responsibilities*- lecturing student, marking and assessment of students, invigilation of examination and supervision of undergraduate students.
2010 -2010	Lecturer in Department of Food Technology, University of Johannesburg, John Orr Building, Doornfontein Campus, Johannesburg South Africa. *Responsibilities*- lecturing Honours' student in Food Technology, marking and assessment of Honours' students, invigilation of examination

Peer-reviewed publications

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Participation in scientific conferences

Oral presentations

1st Mycokey International Conference, Ghent, Belgium, 11-14 September 2017. **"*Fusarium* (Modified) Mycotoxins in Nigerian Sorghum-Based Beer: Natural Occurrence and Influence of Traditional Processing Methods"**. Chilaka, CA.; De Boevre, M.; Atanda, O De Saeger, S.

2nd International Conference on Food Contaminants, Braga, Portugal, 13 – 14 July 2017. **"Investigation of Multi-Mycotoxin Occurrence in Nigerian Cereals using LC-MS/MS".** Chilaka, CA.; De Boevre, M.; Atanda, O De Saeger, S.

39th Mycotoxin Workshop, Bydgoszcz, Poland 2017, 19- 21 June 2017. **"Occurrence and Influence of Processing on *Fusarium* Mycotoxins in Nigerian Traditional Cereal-Based Infant Food"** Chilaka, CA.; De Boevre, M.; Atanda, O De Saeger, S.

8th Trends in Food Analysis symposium, Ghent, Belgium, 24th May 2017. **"Quantification of *Fusarium* Mycotoxins in Nigerian Maize using a Multi-Mycotoxin LC-MS/MS Method"** Chilaka, CA.; De Boevre, M.; Atanda, O; De Saeger, S.

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7th Annual Conference and Workshop of the Mycotoxicology Society of Nigeria, Lagos Nigeria, 2012, 27- 29 June 2012. **"Zearalenone Contamination of South African Commercial Maize and the Cytotoxic Effect of Maize Extracts on Human Lymphocytes"** Chilaka, CA.; De Kock, S.; Dutton, MF.

Poster presentations

5th International Symposium on Mycotoxins and Toxigenic Moulds: Challenges and Perspectives, Ghent, Belgium, 11th May 2016. "**Occurrence of *Fusarium* Mycotoxins and their Modified Forms in Major Cereal Crops and their Processed Product (*Ogi*) from Nigeria**" Chilaka, CA.; De Boevre, M.; Atanda, O; De Saeger, S.

38th Mycotoxin Workshop, Berlin, Germany, 2 – 4 May 2016. "**Incidence of *Fusarium* Mycotoxins in Nigerian Maize"** Chilaka, CA.; De Boevre, M.; Atanda, O; De Saeger, S.

MYCORED AFRICA conference, Cape Town, South Africa, 4 – 6 April 2011. "**Fumonisins in Commercial South African Maize using Different Analytical Techniques**" Chilaka, CA.; Dutton, MF.; Njobeh, PB.; De kock, S.

Courses and workshops

2017	Project Management at Ghent University organised and sponsored by Doctoral School Ghent University, Belgium.
	Communication Skills basics and Conflict Handling organised and sponsored by Doctoral School Ghent University, Belgium.
	Effective Graphical Displays organised and sponsored by Doctoral School Ghent University, Belgium.
2016	Plunge into your own Business Plan at Ghent University organised and sponsored by Doctoral School Ghent University, Belgium.
	Introduction to R - Statistics at Ghent University organised and sponsored by Doctoral School Ghent University, Belgium.
	Effective Scientific Communication organised and sponsored by Doctoral School Ghent University, Belgium.
2015	Advanced Academic English: Conference Skills - Academic Posters organised and sponsored by Doctoral School Ghent University, Belgium.
2014	Intensive Training Programme on Mycotoxin Analysis organised by Laboratory of Food Analysis, Ghent University sponsored by the North Programme of VLIR-UOS (Flemish Interuniversity Council- University Development Cooperation).
	Bread baking course at Dundee College Kingsway Campus, Dundee Scotland United Kingdom.
2012	Urban food and Nutrition Security Course jointly organised by Kasetsart University Thailand and the Food Security Centre of the University of Hohenheim Germany sponsored by the German Academic Exchange Service (DAAD) and hosted by Faculty of Agriculture, Kasetsart University Bangkok, Thailand.